ABILITY OF MEDICAGO SATIVA (ALFALFA) TO REMOVE NICKEL IONS FROM AQUEOUS SOLUTION

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ABSTRACT

The characteristics of the roots and shoots from seven different populations of Medicago sativa (alfalfa) were examined for their ability to bind nickel ions from aqueous solution. Batch laboratory experiments were performed to determine the optimal pH for nickel binding to the alfalfa plant tissues which was between pH 5 and 6. From these experiments, pH profiles were performed to gain information about the chemical functional groups in the alfalfa plant tissues responsible for the nickel binding. Binding-time-dependency studies determined that approximately 80% of the nickel ions bound to the alfalfa plant tissues in less than five minutes. Binding capacity experiments showed that nickel binding was as much as 4.1 mg of nickel per gram of alfalfa biomass. Nickel recovery experiments showed that over 90% of the bound nickel was removed from the alfalfa biomass. Column experiments were conducted to examine the binding of nickel to silica immobilized alfalfa plant tissues under flow conditions. Results from these experiments showed that over 90% of the retained nickel was recovered after 4 bed volumes of 0.1 M HCl solution was passed through the column. After 12 cycles on the same column, the efficiency for nickel removal and recovery from solution was stable.

KEY WORDS

biofiltration, phytoremediation, alfalfa, Medicago sativa, nickel, binding, recovery

INTRODUCTION

As today's technology progresses, the natural environment suffers from the detrimental effects of pollution. The natural process of transportation of metal ions between soil and water consolidates metal contamination in high concentrations that affect the areas of natural ecosystems [1]. Bewley and coworkers studied the effects of heavy metal contamination that get into the environment, by conducting site simulations of smelter contamination [2]. Heavy metal contamination that does get into the environment could cause permanent negative ecological effects [3]. These contaminants can be retained by plants and enter the food chain of animals. Studies have found that cattle which graze on metal contaminated plants will accumulate the toxic metals in their bodies which could then be passed to humans [4]. Therefore, heavy metal contamination of the environment has become an area of increasing concern. Many methods are now being utilized to remove or reduce the metal concentrations in the environment, but most have shown to be somewhat impractical and costly. With the increase in environmental awareness and governmental policies, there has been a push towards devel-

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opment of new, environmentally friendly ways to clean contamination.

Many researchers have investigated methods to prevent or reduce metals in the environment. Biological methods for remediation may provide the answer [5-10]. Many live microbial and fungal systems have been studied and have shown good results [11-13]. Recently, plants have been studied for their ability to remove contaminants from the environment [14-19]. However, dead systems offer many advantages over live systems because they don't fall prey to the toxicological effects of high concentrations of contaminants and can be obtained inexpensively. Dead or inactivated systems may be more practical because they don't require pretreatment with nutrients to maintain the biological activity of the system [20-23]. The immobilization of biomaterial has also proven to be a good method for metal accumulation from contaminated waters under flow conditions [24-26]. Thus, a combination of these methods into a dead or inactivated immobilized system may prove to be very efficient and practical for the removal of metals from contaminated waters.

We chose alfalfa as a source for biomaterial because it has a higher tolerance to concentrated metal contamination than many other plants. Alfalfa has been found to grow near smelters and in fields irrigated with heavy metal contaminated waters [27-28]. The ability of the alfalfa plant to resist the toxicity and accumulate metals may be due to certain compounds in or on the plant tissues. Therefore, alfalfa shows potential for a biomaterial to be used in a dead immobilized system for the removal of metals from contaminated waters. We chose to study seven different populations of alfalfa based on their characteristics. These characteristics may be due to different compounds in each of the populations. Consequently, these different characteristics may influence the binding ability of metals to the alfalfa plant.

Plant	Characteristics
AFRICAN	hot weather tolerant
CW630	drought resistant
FLEMISH	high phosphate content
LADAK	cold weather tolerant
MALONE	mixed variety
MOAPA 69	mixed variety
PERUVIAN	temperate weather

Table 1. Population characteristics.

The objective of our study was to investigate the binding of nickel ions from solution by seven different alfalfa populations. Laboratory batch experiments were performed with ground alfalfa to determine optimal pH and the time required for nickel binding to all the alfalfa biomasses. Capacity experiments were performed to determine the difference of nickel binding in the roots and shoots of the seven populations studied. Column experiments were performed with immobilized alfalfa biomass to examine nickel removal and recovery under flow conditions.

METHODOLOGY

Alfalfa collection

The seven alfalfa populations were selected as representatives from the many different varieties of alfalfa by their individual characteristics (Table 1). The different characteristics of each population may be due to differences in plant composition and may provide different chemical functional groups that could affect nickel binding to the biomasses.

Alfalfa tissues were collected from field studies conducted by Dr. John Henning at New Mexico State University near Las Cruces, New Mexico. Four alfalfa basic germplasms (African, Peruvian, Flemish, Ladak) and two cultivars (Malone, Moapa 69) were

obtained from plots that had received irrigation every 2 weeks during the growing season. One cultivar (Cal West 630) was taken from a dryland test, which received no irrigation. Plants were removed from the soil, washed, and the roots were separated from the shoot material (stems and leaves). All samples were oven dried at 90ºC while Malone and CW630 were also lyophilized. Dried samples were ground to pass through a 100-mesh screen using a Wiley mill.

pH profile studies for nickel binding

Batch laboratory techniques were used for the pH studies. A 250 mg sample of biomass was washed twice with 0.01 M hydrochloric acid (HCl) to remove any debris or soluble biomolecules that might interact with metal ions. Washings were collected, dried and weighed to account for any biomass weight loss. Each biomass sample was resuspended in 50 ml of 0.01 M HCl with tissue concentration approximately 5 mg per ml solution. The pH was adjusted to 2.0, allowed to equilibrate, and 2 ml aliquots of the suspension were transferred into three 5 ml plastic tubes. The pH was then adjusted and allowed to equilibrate at pH 3.0, 4.0, 5.0 and 6.0, and 2 ml aliquot of the suspensions at each pH were transferred into 3 tubes for each pH. The suspensions were centrifuged at 2,500 rpm for 5 minutes and the supernatants were kept for testing to determine if soluble materials were binding the metal. A solution of 0.1 mM nickel nitrate ($Ni(NO_3)_2$) was prepared and pH adjusted to 2.0, 3.0, 4.0, 5.0 and 6.0. At each pH, 2 ml of the nickel solution was added to the respective pH biomass pellet and separated supernatant. In addition, at each respective pH, 2 ml of the 0.1 mM Ni^{2+} solution was transferred to 3 tubes for controls. All the tubes were equilibrated on a rocker for 1 hour. The samples were then centrifuged at 3,000 rpm for 5 minutes and the supernatants for the pellets were transferred to clean respective tubes. Final pHs for all tubes were recorded and analysis for nickel was performed by flame atomic absorption.

Time dependence studies for nickel binding

A 500 mg sample of biomass was washed twice with 0.01 M HCl to remove any debris or soluble biomolecules that might interact with metal ions. The washings were collected, dried and weighed to account for any biomass weight loss. Each biomass sample was resuspended in 100 ml of deionized water with tissue concentration approximately 5 mg per ml solution. The solution was then adjusted to pH 5.0 and allowed to equilibrate. Two ml of the suspension was transferred to 24 tubes; 3 tubes for each time interval of 5, 10, 15, 20, 25, 30, 45 and 60 minutes. After centrifugation, 2 ml of 0.1 mM nickel solution was added to each of the tubes and controls. All the tubes were equilibrated by rocking and were removed at the appropriate time intervals. The samples were then centrifuged at 3,000 rpm for 5 minutes and the supernatants from the pellets were transferred to clean respective tubes. Final pHs for all tubes were recorded, and analysis for nickel was performed by flame atomic absorption.

Nickel binding capacity studies

Samples of 100 mg of biomass were washed twice with 0.01 M HCl, and washings were collected and weighed to determine biomass loss. Washed biomass was resuspended in 20 ml of deionized water and pH adjusted to 5.0. Two ml of the suspension was transferred to 3 tubes and then centrifuged. The supernatants were saved for testing. Two ml of 0.3 mM Ni^{2+} solution was added to each of the tubes and controls and were equilibrated for 10 minutes. After centrifugation, the supernatants were saved for analysis and again 2 ml of 0.3 mM nickel solution was added. This was repeated 12 times or until the

saturation point was achieved and a final pH for all tubes was recorded. Samples were diluted as required to stay within the calibration linear range and analysis for nickel was performed by flame atomic absorption.

Desorption of the adsorbed nickel

Pellets from capacity studies with adsorbed nickel were exposed to 2 ml of 0.1 M HCl, equilibrated by rocking for 5 minutes, and then centrifuged. Supernatants were collected for analysis and diluted as required to stay within the calibration range. Pellets were then exposed to 2 ml of 1 M HCl to strip any remaining metal and equilibrated by rocking for 5 minutes. After centrifugation, the supernatants were analyzed. All analysis for nickel was performed by flame atomic absorption.

Immobilization of alfalfa biomass

The method for immobilization of cell wall material within a polysilicate matrix was similar to that reported by Rayson and coworkers [26]. A 5 g sample of biomass was washed twice by vortexing the sample with water and was centrifuged for five minutes at 3,000 rpm. This step will remove solubles and debris. Next, 75 ml of 5% H_2SO_4 was mixed with enough 6% Na_2SiO_3 solution to raise the pH to 2.0. At pH 2.0, the 5 g of washed biomass was added to the silica solution and allowed to stir for 15 minutes. The pH was then raised slowly by addition of 6% sodium silicate (Na_2SiO_3) to reach a final pH of 7.0. The polymer gel was washed with water enough times so that by the addition of two drops of barium chloride ($BaCl_2$) there was no white precipitate forming. $BaCl_2$ was used to indicate whether the sulfates had been removed. The polymer gel with the immobilized biomass was dried overnight at 60ºC and then ground by mortar and pestle and sieved to 20-40 mesh size.

Column experiments

One bed volume equals the volume of immobilized biomass inside the column. Six ml of the immobilized alfalfa was used in the column. The column was washed with 10 bed volumes of 0.01 M sodium acetate buffer at pH 5.0 and the effluent pH was checked to ensure that the column was at the optimal binding pH. A flow rate of 1 ml per minute was used to pass 120 bed volumes of 5.0 ppm Ni^{2+} solution in 0.01 M sodium acetate at pH 5.0. Each bed volume was collected and analyzed by flame atomic absorption.

Recovery of nickel from column

To remove the bound nickel, 0.01 M HCl was passed at a flow rate of 1 ml per minute. Each bed volume was collected and analyzed by flame atomic absorption as indicated.

Analytical procedure

Analysis for nickel was performed using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. Impact bead was utilized to improve the sensitivity at a wavelength of 352.4 nm. Samples were read three times, and a mean value and relative standard deviation was computed. Calibrations were performed in the range of analysis, and a correlation coefficient for the calibration curve of 0.98 or greater was obtained. The instrument response was periodically checked with known nickel standards. The difference between the initial metal concentration and the remaining metal concentration in effluents was assumed to be taken up by the biomass.

Data analysis

The experiments were performed in triplicate and the samples was analyzed in triplicate. For each set of given data, standard statistical methods were used to determine the mean values and standard deviations.

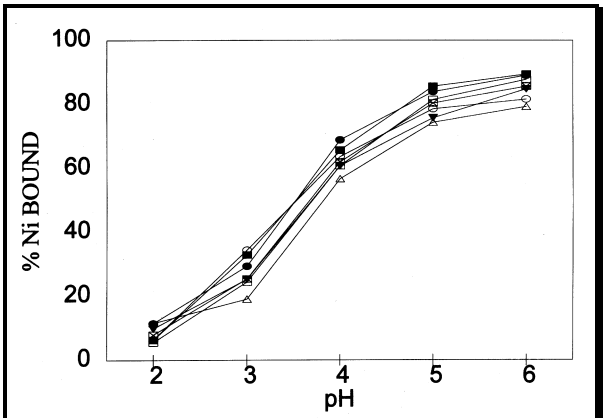

Figure 1. Effect of pH on nickel binding by different populations of alfalfa roots (African θ, CW 630 ■, Flemish ●, Ladak ▼, Malone □, Moapa 69 △ , Peruvian ☒). The biomass (5 mg/ml) was shaken for 1 hour at the appropriate pH with 0.1 mM nickel.

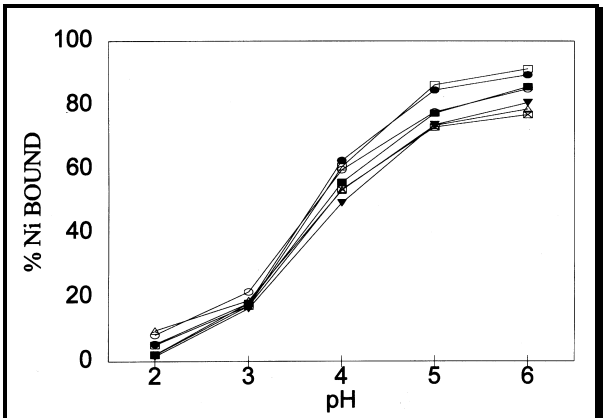

Figure 2. Effect of pH on nickel binding by different populations of alfalfa shoots (African θ, CW 630 ■, Flemish ●, Ladak ▼, Malone □, Moapa 69 △, Peruvian ☒). The biomass (5 mg/ml) was shaken for 1 hour at the appropriate pH with 0.1 mM nickel.

Confidence intervals of 95% were calculated for each set of samples to determine the error margin.

RESULTS AND DISCUSSION

The pH studies performed on the seven different populations showed that binding of nickel to the alfalfa biomass is pH dependent. Figures 1 and 2 show the binding of nickel ions to the various alfalfa roots and shoots as the pH is raised from 2.0 to 6.0. For all the varieties tested, 85% to 95% binding of nickel ions occurred between pH 5.0 and 6.0. From Figures 1 and 2, it can be observed that as the pH decreased the binding of nickel ions to the alfalfa biomass also decreased. This same trend in pH-dependent binding was observed for copper binding to all the same seven populations studied [29]. This trend observed in pH-dependent binding might be due to an ion-exchange type of binding mechanism as proposed by other researchers [21]. This trend in pH dependency also suggests that carboxyl groups may be involved in the nickel binding to the alfalfa biomass. The ionization constants (pKs) values for different carboxyl groups have been reported to be around 4-5 [30, 31]. When the pH is higher than 3-4, the carboxyl groups are deprotonated and left with a negative charge. Therefore at pHs above 3-4, the negatively charged carboxylate groups may attract the positively charged nickel ions, consequently binding and removing the nickel ions from solution. At pHs lower than 3-4, the carboxyl groups become protonated and no longer attract the positively charged nickel ions. Figures 1 and 2 show very little binding of nickel ions as the pH decreases below the 3-4 range. From this unique feature of low binding at low pH, we hypothesized that by lowering the pH, we may be able to recover the nickel ions from the alfalfa biomass. Experiments were conducted to investigate this possibility and are reported herein.

Time dependency experiments were conducted in order to determine how long the alfalfa biomass would take to bind the nickel ions at optimal pH. Since all soluble materials were eliminated during prior washings, the binding could only have occurred by the alfalfa biomass. Figure 3 shows the binding time for nickel by the roots for all the populations studied, and Figure 4 shows the binding time for nickel by the shoots for all the populations studied. As can be seen in the figures, nickel bound to the alfalfa biomass in less than five minutes. Not only was the binding of the nickel ions to the biomass rapid, but it

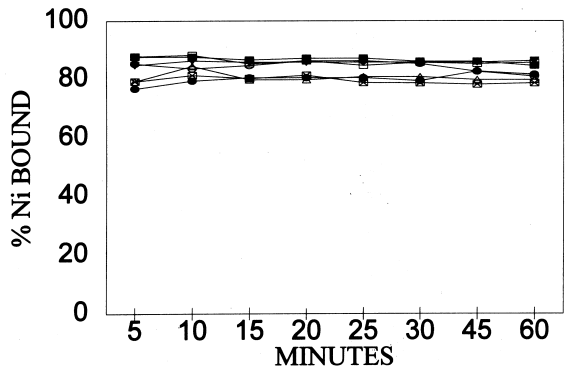

Figure 3. Time dependency studies for nickel binding by alfalfa roots (African θ, CW 630 ■, Flemish ●, Ladak ▼, Malone □, Moapa 69 △, Peruvian ☒). The biomass was shaken for the appropriate time with 0.32 mM nickel.

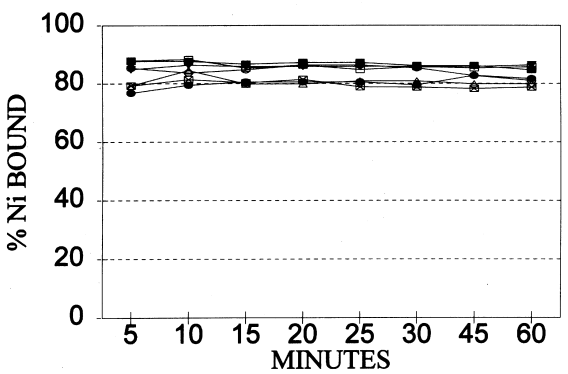

Figure 4. Time dependency studies for nickel binding by alfalfa roots (African θ, CW 630 ■, Flemish ●, Ladak ▼, Malone □, Moapa 69 △, Peruvian ☒). The biomass was shaken for the appropriate time with 0.32 mM nickel.

also remained stable. After the biomass was equilibrated with the same nickel solution for one hour, the amount of nickel ions bound to the alfalfa biomass did not decrease. Since the nickel ions were not washed off from the biomass physically by equilibration, the chemical interaction between the nickel ions and the binding sites is stable. This trend in rapid and stable binding was also observed for copper binding with all the populations studied [29]. The rapid binding of nickel ions from solution could mean that the binding sites for nickel are located on the alfalfa plant cell walls, and the nickel ions are not being taken into the cell. Similar conclusions were obtained by Zhang and Majidi who conducted in vivo ^{31}P NMR studies with Stichlococcus bacillaris [32]. They found that Cu^{2+}, Zn^{2+}, Cd^{2+} and Mn^{2+} bound rapidly on the cell walls. Since the alfalfa plant tissues were inactivated, binding of the nickel ions to the cell walls should not be due to a biological process, but instead by chemical binding to functional groups such as carboxylates.

Population		mg Nickel/g Biomass
AFRICAN	(ROOTS)	1.80 ± 0.28
	(SHOOTS)	4.11 ± 0.40
CW630	(ROOTS)	2.65 ± 0.10
	(SHOOTS)	1.60 ± 0.10
FLEMISH	(ROOTS)	1.80 ± 0.10
	(SHOOTS)	2.05 ± 0.48
LADAK	(ROOTS)	1.61 ± 0.80
	(SHOOTS)	1.90 ± 0.09
MALONE	(ROOTS)	1.80 ± 0.10
	(SHOOTS)	2.20 ± 0.14
MOAPA	(ROOTS)	1.80 ± 0.10
	(SHOOTS)	2.40 ± 0.30
PERUVIAN	(ROOTS)	1.90 ± 0.20
	(SHOOTS)	1.41 ± 0.10

Note: 95% confidence interval was used to determine error.

Table 2. Capacity for nickel uptake by different alfalfa populations.

Binding capacity experiments were performed at pH 5.0 with the roots and shoots of the seven different populations of alfalfa to determine the amount of nickel ions the biomass could bind. Table 2 shows the binding capacities for the different populations in milligrams of nickel bound per gram of biomass. The majority of the shoots showed to have more nickel binding than the roots with the exception of CW630 and Peruvian. This phenomena may be due to a difference in composition and chemical functional groups in the plants roots and shoots. Since CW630 is a drought-tolerant variety, it may have evolved modified com-

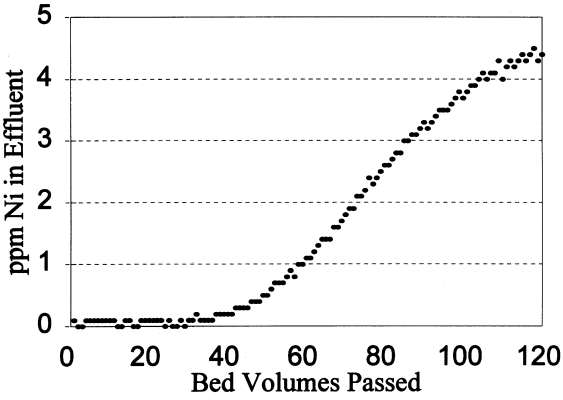

Figure 5. Amount of nickel remaining in bed volumes collected after a solution of 5 ppm nickel at pH 5.0 was passed through a column of immobilized Malone shoots.

ponents in the roots. African shoots bound the most nickel for all the populations studied, and this correlates with data observed with copper binding.

After the alfalfa biomass was saturated with nickel during the capacity experiments, we were interested in testing our hypothesis for removing the bound nickel. Theoretically, by lowering the pH below 3-4, the protons would displace the nickel ions. Therefore, we treated the nickel saturated alfalfa biomass with low concentrated acid solution in an attempt to displace the bound nickel ions. By using low strength acid the biomass should not be destroyed and could be reused again. Table 3 shows the percentage of nickel ions recovered from the seven different populations of alfalfa. The majority of the populations showed over 80% recovery with African shoots being the highest at 97.7%. The alfalfa biomasses that did have high recovery percentages may be due to higher concentrations of binding sites that interact with protons more easily and displace the bound nickel ions. We believe that carboxyl groups may be involved.

Populations		% Nickel Recovered
AFRICAN	(ROOTS)	93.11% ± 3.79
	(SHOOTS)	97.67% ± 10.82
CW630	(ROOTS)	74.64% ± 11.20
	(SHOOTS)	70.48% ± 13.14
FLEMISH	(ROOTS)	97.53% ± 13.14
	(SHOOTS)	97.33% ± 12.25
LADAK	(ROOTS)	97.42% ± 5.17
	(SHOOTS)	83.60% ± 7.58
MALONE	(ROOTS)	85.41% ± 8.72
	(SHOOTS)	84.85% ± 16.53
MOAPA	(ROOTS)	86.73% ± 5.17
	(SHOOTS)	78.19% ± 2.48
PERUVIAN	(ROOTS)	80.39% ± 6.25
	(SHOOTS)	74.79% ± 8.95

Note: 95% confidence interval was used to determine error.

Table 3. Desorption of nickel with 0.1 M HCl.

The batch laboratory experiments showed that alfalfa has the ability to bind nickel ions and remove them from solution, but a batch system would not be practical for removing nickel ions from contaminated waters. Therefore, column experiments were performed to study the binding of nickel ions to the alfalfa biomass under flow conditions. In order to perform these studies it was necessary to use a solid support for the alfalfa biomass to prevent column clogging and to help maintain optimal flow through the column. It was also important to chose a support material that would not add to the pollution problem. A silica support material was chosen because it would not leach any harmful reagents if decomposed and was biodegradable along with the biomass. The alfalfa biomass was immobilized in a polysilicate matrix and ground into 20-40 mesh size particles to be packed into the columns. Figure 5 shows the amount of nickel ions that remained in the effluent after a solution of 5 ppm nickel at pH 5.0 was passed through a column of immobilized Malone shoots. It can be seen that most of the nickel ions were retained in the column until bed volume 40. After bed volume 40, the column slowly decreased in its ability to remove the nickel ions from solution. This may be due to the saturation of the binding

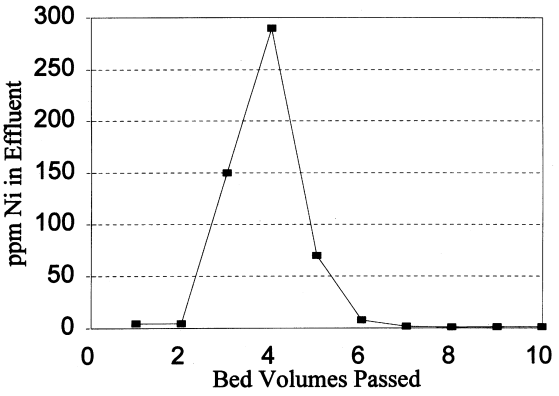

Figure 6. Amount of nickel removed per bed volume of 0.1 m HCl solution passed through a column of immobilized Malone shoots.

sites by the nickel ions. At bed volume 120, the column was nearly saturated and was able to bind very little nickel.

Since the column of immobilized Malone shoots was nearly saturated and would no longer bind nickel ions efficiently, we wanted to determine if the nickel ions could be removed from the column by addition of low concentrated acid. Figure 6 shows the amount of nickel ions recovered from the column per bed volume of low concentrated acid passed. It can be seen that nearly all of the nickel ions were removed in two bed volumes of low concentrated acid. Approximately 90% of the bound nickel was recovered. The same column was used again to remove nickel ions from solution to determine if the acid had any effect on the immobilized biomass. After 12 cycles of removal and recovery the column was still efficient in its ability to bind nickel ions from solution. Further experiments are being performed in our laboratory to determine the binding of several different metal ions by the different populations of alfalfa. We will also be conducting interference studies to determine what effects cations and anions will have on the metal binding under flow conditions.

CONCLUSIONS

The characterization of the nickel ion binding to the different alfalfa populations showed that the binding mechanism is pH-dependent and also occurs in less than five minutes. The binding of the nickel ions may be on the cell wall of the alfalfa plant tissues. Alfalfa biomass that was saturated with nickel ions shows the remarkable ability for nickel recovery by treatment with low concentrated acid. Through column experiments, we showed that by using immobilized Malone shoots we were successful in removing and recovering nickel ions from solution. Not only was the column successful in nickel binding, but it was also reusable. These studies show that immobilized alfalfa has the potential to be used as a biofilter for removal and recovery of nickel ions from contaminated waters. Not only is the alfalfa inexpensive, its also practical. This innovative technology provides a reusable material that is not only biodegradable, but it is also environmentally friendly.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the University of Texas at El Paso's Center for Environmental Resource Management (CERM) through funding from the Office of Exploratory Research of the U.S. Environmental Protection Agency (cooperative agreement CR-819849-01-4). We also acknowledge Mr. Monte Mauldin from the Department of Chemistry at New Mexico State University for his contributions to this project.

REFERENCES

1. D.D. Runnells, T.A. Shepherd and E.E., Metals in Water Determining Natural Background Concentrations in Mineralized Areas, Environ. Sci. Technol., 26 (1992) 2316-2323.

2. R.J.F. Bewley, Effect of Heavy Metal Pollution on Oak Leaf Microorganism,

App. Enviro. Microbiol., 40 (1980) 1053-1059.

3. G. Micera and A. Dessi, Chromium Adsorption by Plant Roots and Formation of Long-Lived Cr(V) Species: An Ecological Hazard?, J. Inorg. Biochem., 34 (1988) 157-166.

4. W.F. Chamberlain and J.A. Miller, Barium in Forage Plants and the Manure of Cattle Treated with Barium, J. Agri. Food Chem., 30 (19982) 463-465.

5. R.M. Atlas, Bioremediation, Chem. Eng. News, 3 (1995) 32-42.

6. W.J. Jewell, Resource-Recovery Wastewater Treatment, American Scientist, 82 (1994) 366-375.

7. D.A. Mays, P.M. Giordano and A.D. Behel Jr., Impact of Fluidized Bed Combustion Waste on Metal Content of Crops and soil, Water, Air, and Soil Pollution, 57-58 (1991) 307-317.

8. H. Tan, J.T. Champion, J.F. Artiola, M.L. Brusseau and R.M. Miller, Complexation of Cadmium by a Rhamnolipid Biosurfactant, Enviro. Sci. Technol., 26 (1994) 2402-2406.

9. C.R.N. Rao, L. Iyengar and C. Venkobachar, Sorption of Copper(II) from Aqueous Phase by Waste Biomass, J. Environ. Eng., 119 (1993) 369-377.

10. K.L. Godtfredsen and A.T. Stone, Solubilization of Manganese Dioxide-Bound by Naturally Occurring Organic Compounds, Environ. Sci. Technol., 28 (1994) 1450-1458.

11. J. Bender, S. Rodriguez-Eaton, U.M. Ekanemesang and P. Phillips, Characterization of Metal-Binding Bioflocculants Produced by the Cyanobacterial Component of Mixed Microbial Mats, App. Environ. Microbiol., 60 (1994) 2311-2315.

12. C. Cervantes and F. Gutierrez-Corona, Copper Resistance Mechanisms in Bacteria and Fungi, FEMS Microbiol. Rev. 14 (1994) 121-138.

13. R. El-Aziz, J.S. Angle and R.L. Chaney, Metal Tolerance of Rhizobium meliloti Isolated from Heavy-Metal Contaminated Soils, Soil Biol. Biochem., 23 (1991) 795-798.

14. V. Dushenkov, P.B.A.N. Kumar, H. Motto and I. Raskin, Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams, Environ. Sci. Technol., 29 (1995) 1239-1245.

15. P.B.A.N. Kumar, V. Dushenkov, H. Motto and I. Raskin, Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils, Environ. Sci. Technol., 29 (1995) 1232-1238.

16. A.J.M. Baker, R.D. Reeves and A.S.M. Hajar, Heavy Metal Accumulation and Tolerance in British Populations of the Metallophyte Thlaspi caerulescens, J. & C. Presl (Brassicaceae), New Phytol., 127 (1994) 61-68.

17. C.D. Scott, Removal of Dissolved Metals by Plant Tissue, Biotechnol. Bioeng., 39 (1992) 1064-1068.

18. H. Lue-Kim and W.E. Rauser, Partial Characterization of Cadmium-Binding Protein from Roots of Tomato, Plant Physiol., 81 (1986) 896-900.

19. V. Romheld and H. Marschner, Mechanism of Iron Uptake by Peanut Plants, Plant Physiol., 71 (1983) 949-954.

20. T. Viraraghavan and M.M. Dronamraju, Removal of Copper, Nickel and Zinc from Wastewater by Adsorption Using

Peat, J. Environ. Sci. Health, A26 (1993) 1261-1276.

21. J.R. Lujan, D.W. Darnall, P.C. Stark, G.D. Rayson and J.L. Gardea-Torresdey, Metal Ion Binding by Algae and Higher Plant Tissues: A Phenomenological Study of Solution pH Dependence, Solvent Extraction and Ion Exchange, 12 (1994) 803-816.

22. H.D. Ke, E.R. Birnbaum, D.W. Darnall and G.D. Rayson, Characterization of the Carboxyl Groups on Datura innoxia Using Eu(III) Luminescence, Environ. Sci. Technol., 26 (1992) 782-788.

23. T.O. Odozi, S. Okeke and R.B. Lartey, Studies on Binding Metal Ions with Polymerized Corn Cob and a Composite Resin with Sawdust and Onion Skin, Agricultural Wastes, 12 (1985) 13-21.

24. G.J. Ramelow, L. Liu, C. Himel, D. Franlick, Y. Zhao and C. Tong, The Analysis of Dissolved Metals in Natural Water after Preconcentration on Biosorbents of Immobilized Lichen and Seaweed Biomass in Silica, Inter. J. Anal. Chem., 53 (1993) 219-232.

25. L. De Rome and G.M. Gadd, Use of Pelleted and Immobilized Yeast and Fungal Biomass for Heavy Metal and Radionuclide Recovery, J. Ind. Microbiol., 7 (1991) 97-104.

26. H.D. Kee and G.D. Rayson, Luminescence Study of UO_2^{2+} Binding to Immobilized Datura innoxia Biomaterial, App. Spectroscopy, 47 (1993) 44-51.

27. R. Carrillo G. and L.J. Cajuste, Heavy Metals in Soils and Alfalfa (Medicago sativa L.) Irrigated with Three Sources of Wastewater, J. Environ. Health, A27 (1992) 1771-1783.

28. L.J. Cajuste, R. Carrillo G., E. Cota G. and R.J. Laird, The Distribution of Metals from Wastewater in the Mexican Valley of Mezquital, Water, Air, and Soil Pollution, 57-58 (1991), 763-771.

29. J.L. Gardea-Torresdey, K.J. Tiemann, J.H. Gonzalez, J.A. Henning and M.S. Townsend, Uptake of Copper Ions from Solution by Different Populations of Medicago sativa (Alfalfa), Solvent Extraction and Ion Exchange (1995) submitted.

30. I.H. Segel, Biochemical Calculations 2nd Edition, John Wiley and Sons Inc., New York, 1976.

31. S. Hunt, In: H. Eccles and S. Hunt (Eds.), Immobilization of Ions by Biosorption, Ellis Horwood Limited, Chichester, England, 1986.

32. W. Zhang and V. Majidi, Monitoring the Cellular Response of Stichococcus bacillaris to exposure of Several Different Metals Using in Vivo ^{31}P NMR and Other Spectroscopic Techniques, Environ. Sci. Technol., 28, (1994) 1577-1581.