

Removal of nickel ions from aqueous solution by biomass and silica-immobilized biomass of *Medicago sativa* (alfalfa)

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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 5 min. 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 more than 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 more than 90% of the retained nickel was recovered after four bed volumes of 0.1 M HCl solution were passed through the column. After 12 cycles on the same column, the efficiency for nickel removal and recovery from solution was stable.

Keywords: Biofiltration; Phytoremediation; Alfalfa; *Medicago sativa*; Nickel; Binding; Recovery

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1. 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 the 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 subsequently 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 unpractical and costly. With the increase in environmental awareness and governmental policies, there has been a push toward development 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 biologic activity of the organism [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 the result of 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 caused by different compounds in each of the populations. Consequently, these different characteristics may influence the binding ability of toxic hazardous metal ions such as nickel to the alfalfa plant.

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.

2. 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 caused by 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 two 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 90°C 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, 6.0, and 2 ml aliquots of the suspensions at each pH was transferred into three tubes for each pH. The suspensions were centrifuged at 2,500 rpm for 5 min. and the supernatants were kept for testing to determine if soluble materials were binding the metal. A solution of 0.1 mM Nickel Nitrate ($\text{Ni}(\text{NO}_3)_2$) (equal to 5.9 ppm nickel) was prepared and pH

Table 1
Population characteristics of the alfalfa varieties

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

adjusted to 2.0, 3.0, 4.0, 5.0, and 6.0. At each pH, 2 ml of the nickel solution were 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 were transferred into three tubes for controls. All the tubes were equilibrated on a rocker for 1 h. The samples were then centrifuged at 3,000 rpm for 5 mins 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 spectroscopy.

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. 2 ml of the suspension were transferred into 24 tubes; 3 tubes for each time interval of 5, 10, 15, 20, 25, 30, 45, and 60 mins. After centrifugation, 2 ml of 0.1 mM nickel solution were 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 mins and the supernatants from the pellets were transferred into 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. 2 ml of the suspension were transferred into three tubes and then centrifuged. The supernatants were saved for testing. 2 ml of 0.3 mM Ni^{2+} solution (equal to 17.7 ppm nickel) were added to each of the tubes and controls and were equilibrated for 10 mins. After centrifugation, the supernatants were saved for analysis and again 2 ml of 0.3 mM nickel solution were 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 five 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 mins. After centrifugation, the supernatants were analyzed. All analyses for nickel were 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 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 were mixed with enough 6% sodium silicate (Na_2SiO_3) solution to raise the pH to 2.0. At pH 2.0, the 5 g of washed biomass were added to the silica solution and allowed to stir for 15 mins. The pH was then raised slowly by

addition of 6% sodium silicate 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 60°C and then ground by mortar and pestle and sieved to 20–40 mesh size.

Column Experiments. One bed volume equals the volume of actual immobilized biomass inside the column. 6 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.

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 were analyzed in triplicate. For each set of given data, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples to determine the error margin.

3. Results and discussion

The pH studies performed on the seven different populations showed that binding of nickel to the alfalfa biomass is pH dependent. Fig. 1 and Fig. 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 Fig. 1 and Fig. 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 the result of 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 nickel binding to the alfalfa biomass. The values of the ionization constants (pK_as) 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

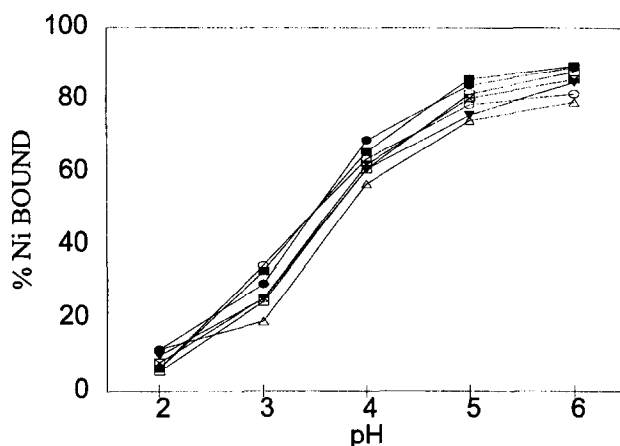


Fig. 1. Effect of pH on nickel binding by different populations of alfalfa roots. (African \ominus , CW 630 \blacksquare , Flemish \bullet , Ladak \blacktriangledown , Malone \square , Moapa 69 \triangle , Peruvian \boxtimes). The biomass (5mg/ml) was shaken for 1 h at the appropriate pH with 0.1 mM nickel.

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. Fig. 1 and Fig. 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 hypothesize 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.

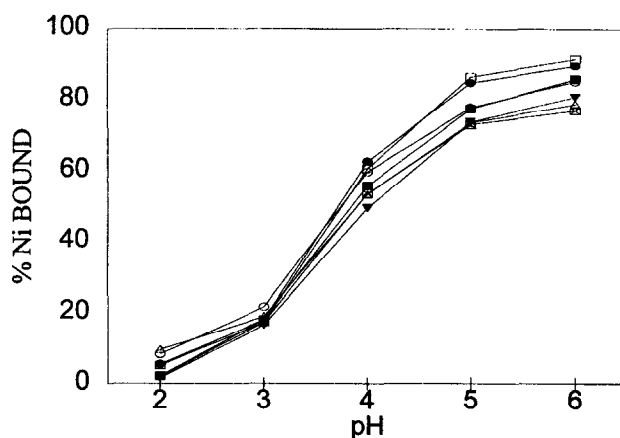


Fig. 2. Effect of pH on nickel binding by different populations of alfalfa shoots. (African \ominus , CW 630 \blacksquare , Flemish \bullet , Ladak \blacktriangledown , Malone \square , Moapa 69 \triangle , Peruvian \boxtimes). The biomass (5mg/ml) was shaken for 1 h at the appropriate pH with 0.1 mM nickel.

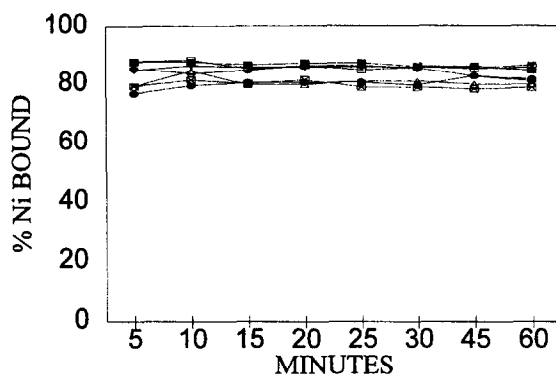


Fig. 3. Time dependency studies for nickel binding by alfalfa roots. (African ○, CW 630 ■, Flemish ●, Ladak ▼, Malone □, Moapa 69 △, Peruvian ⊠). The biomass was shaken for appropriate time with 0.32 mM nickel.

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. Fig. 3 shows the binding time for nickel by the roots for all the populations studied, and Fig. 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 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

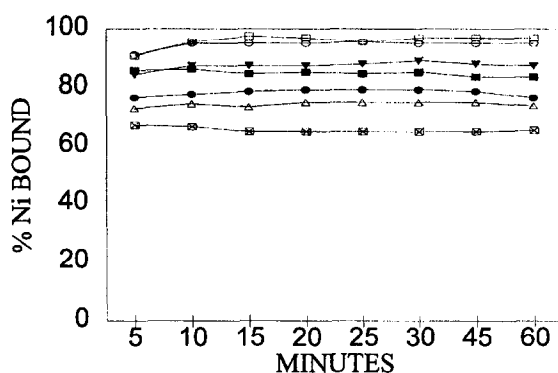


Fig. 4. Time dependency studies for nickel binding by alfalfa shoots. (African ○, CW 630 ■, Flemish ●, Ladak ▼, Malone □, Moapa 69 △, Peruvian ⊠). The biomass was shaken for appropriate time with 0.32 mM nickel.

Table 2
Capacity for nickel uptake by different alfalfa populations

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.

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 *Stichococcus 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.

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 exception of CW630 and Peruvian. These phenomena may be the result of 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 components 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 a 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 more than 80% recovery with African shoots being the highest at 97.7%. The alfalfa biomasses that did have high recovery percentages may possess higher concentrations of binding sites that interact with protons more easily and

Table 3
Desorption of nickel with 0.1M HCl

	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.

displace the bound nickel ions. We believe that carboxyl groups may be the main functionalities involved in nickel binding.

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 choose a support material that would not

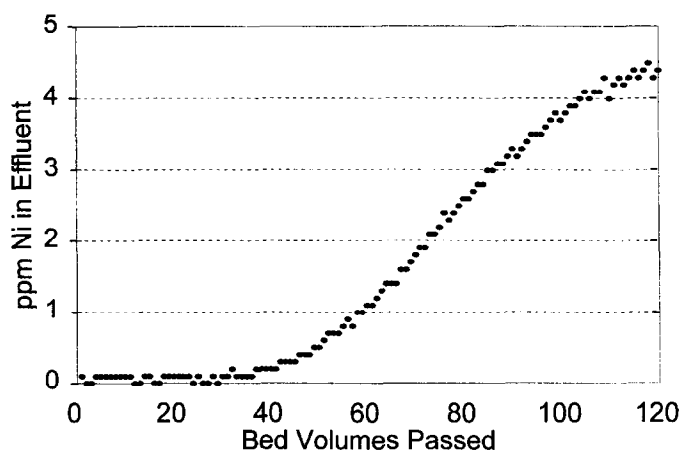


Fig. 5. Concentration of nickel remaining as a function of number of bed volumes collected for a solution of 5 ppm nickel at pH 5.0 exiting a column of immobilized malone shoots.

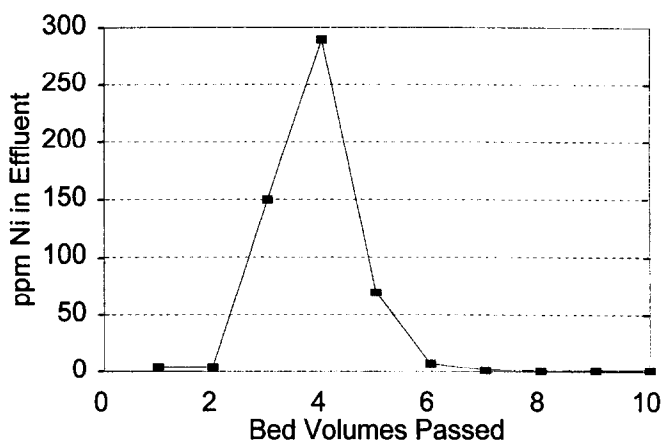


Fig. 6. Amount of nickel removed per bed volume of 0.1 M HCl solution passed through a column of immobilized malone shoots.

add to the pollution problem. A silica support material was chosen because it would not leach any harmful reagents if decomposed and along with the biomass was biodegradable. The alfalfa biomass was immobilized in a polysilicate matrix and ground into 20–40 mesh size particles to be packed into the columns. Fig. 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 because of the saturation of the binding 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. Fig. 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 affect on the immobilized biomass. After 12 cycles of removal and recovery the column still retained 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.

4. Conclusions

The characterization of nickel ion binding to the different alfalfa populations showed that the binding mechanism is pH dependent and also occurs in less than 5 mins. 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 binding nickel 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 alfalfa inexpensive, it is also widely available. This innovative technology provides a reusable material that is not only biodegradable, but it is also environmentally friendly.

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