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Use of hop (*Humulus lupulus*) agricultural by-products for the reduction of aqueous lead(II) environmental health hazards

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Abstract

The agricultural by-products of the hop plant (*Humulus lupulus* L.) were investigated to determine their potential for use in the removal of heavy lead(II) ions from contaminated aqueous solutions. Separate batch laboratory experiments were performed to establish the optimal binding pH, time exposures, and capacity of the metal adsorption for lead(II) ions by dried and ground hop leaves and stems biomass. Results from these studies have shown a pH dependent binding trend from pH 2–6, with optimum binding occurring around pH 5.0. Time dependency experiments showed a rapid adsorption of lead(II) ions within the first 5 min of contact. Binding capacity experiments demonstrated that 74.2 mg of lead(II) were bound per gram of leaf biomass. Similarly overall capacity was seen for the leaves and stems. Desorption of 99% of the bound lead(II) ions was achieved by exposing the metal laden biomass to 0.5 M sodium citrate. Further experiments were performed with silica-immobilized hop tissues to determine the lead(II) binding ability under flow conditions. Comparison studies were performed with ion-exchange resins to evaluate the binding ability and to gain further insight into the metal binding mechanism. X-ray absorption spectroscopy experiments were also utilized to gain further insight into the possible lead(II) binding mechanism by the hop plant tissue. Results from these studies indicate that carboxyl ligands are involved in the binding of lead(II) from aqueous solution. These findings show that the use of hop agricultural waste products may be a viable alternative, for the removal and recovery of aqueous lead(II) ions from contaminated waters.

Keywords: Bioremediation; Phytoremediation; Hops; Agricultural by-products; Lead binding

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1. Introduction

Lead is among several toxic heavy metals, that present a tough environmental problem when found as a contaminate in soil or water [1]. Frequently toxic metals such as lead are found in sewage or industrial effluents. Since these metals do not degrade through natural processes and can accumulate in the ecosystem over time, they need to be removed from natural and industrial effluents [2]. Traditional methods for heavy metal removal from contaminated waters involve filtration, flocculation, activated charcoal, and ion-exchange resins, which are costly and can result in toxic exposures of the workers involved [3–5]. Therefore, new cost effective and environmentally friendly methods need to be developed, which are easily implemented to clean lead and other toxic metals from contaminated areas and industrial waste waters.

Several researchers have shown that biological systems such as micro-organisms, fungi and algae can adsorb lead and other toxic metal ions from contaminated waters [6–13]. Among these novel biological systems is phytoremediation, which is the application of plants to reduce the toxic metals and other contaminants. Many researchers have found that plants are able to absorb metal contaminants directly into their tissues and thus remove the toxic elements from the environment (phytoremediation) [14–16]. However, one drawback of phytoremediation is that it requires extended growth periods. Nevertheless, the plants used for phytoremediation may still possess a solution to remove lead and other toxic metal ions from contaminated waters. Phytofiltration is usually described as the use of plant tissues, typically stems and leaves, to adsorb pollutants (mainly metals) from water and aqueous waste streams. Researchers have found that biomaterials derived from a variety of plants, including legumes and desert plants are able to adsorb cadmium, copper, chromium, nickel, lead and zinc ions from aqueous solutions [17–28]. The overall benefits of phytofiltration is lower cost for ground water and end-of-pipe treatment, which is applicable to a broad range of metals, with minimal environmental disturbance. However, the ideal source for the plant tissue would be an agricultural waste by-product or some sort of mass-produced biomaterial that has little or no value. This will not only reduce the cost of a plant tissue based filtration system, but it will also provide value to a plant by-product for recycling waste.

The hop plant (*Humulus lupulus* L.) may be a good source of plant material for the removal of toxic heavy metal ions from aqueous solutions. Hop is a perennial viniferous plant that grows in excess of 6–7 m per growing season [29]. The harvested portion of the plant is the mature female inflorescence, or cone, which is commonly used in the brewing (flavinoids) and pharmaceutical industries [30–32]. Leaf and stem materials are typically burned or land filled as a means of disposal following harvest of the cone. Unfortunately, leaf and stem material make up the vast majority of the biomass produced by hop (approximately 75% of biomass) [33–36]. Thus, some means of alternative use for the leaf and stem materials would be highly desirable. Since researchers have found that live plants of *H. lupulus* are able to accumulate metals such as copper, iron, zinc and nickel from the soil, it may posses the natural binding sites, necessary to be used as a biomaterial for phytofiltration [37,38].

The objective of this study, is to determine if the agricultural by-products (leaf and stem materials) from hop cultivation can be used to remediate contaminated aqueous solutions. Batch laboratory methods were employed to investigate the potential for lead(II) adsorption

by dried and ground hop leaves and stems biomass. Conditions were varied to determine the optimal binding pH, exposure time, and adsorption capacity for lead(II) binding. In addition, desorption experiments were carried out to determine if the bound lead(II) ions could be recovered from the metal laden biomass. In order to better understand the actual binding mechanisms involved in the metal ion adsorption, batch laboratory experiments were compared with cation exchange resins and further experiments were performed by X-ray absorption spectroscopy (X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS)) of the lead laden biomass. The information gained from these studies will indicate whether the biomass of hop leaves and stems has the potential to be used for the removal and recovery of lead(II) ions from contaminated hazardous solutions.

2. Methodology

2.1. Hop (Humulus lupulus L.) collection

Hop variety used for this study was grown at the USDA-ARS Hop Research Farm located outside of Corvallis, Oregon. The soil type for this region is a Xeric Mesic Soil (Dayton-Amity-Concord). Plants were allowed to grow to cone maturity and then cut down. Leaves and stems were washed with de-ionized (D.I.) water and allowed to air dry. Then, the leaf material was separated from stem material prior to the final drying, which was accomplished in a forced air dryer set at 65 °C. Stem materials were cut into 0.3 m sections before grinding. All grinding was accomplished using a Wiley-Mill and the resulting powder was sieved with a 100 mesh screen to achieve uniform particle size.

2.2. Determination of total carbon, nitrogen and sulfur in hop samples

The technique used for the determination is based on the quantitative "dynamic flash combustion" method, where the samples are maintained at 1020 °C in an enriched oxygen atmosphere, where even thermally resistant substances are completely oxidized. In short, quantitative determinations were achieved by passing the resulting mixture of gases over a copper catalyst layer, to remove the excess oxygen and subsequently analyzed using ion chromatography. The instrument was calibrated with known standard compounds using the linear regression method. The samples were analyzed three times and the mean values and standard deviations were determined.

2.3. pH profile studies for lead binding

Batch laboratory methods were carried out based on the similar procedures previously described [19–23]. A 250 mg sample of hops biomass (either leaves or stems) was weighed and washed twice with 0.1 M HCl to remove any soluble biomolecules or possible debris that might interact with the lead(II) ion binding. The washings from the hop plant tissues were collected and dried to account for any biomass loss during washing. The hops biomass was then suspended in 50 ml of 0.01 M HCl (biomass concentration of 5 mg/ml), adjusted to pH of 2.0, and allowed 2 ml to equilibrate. Two milliliters of the hops biomass suspension (10 mg

biomass) were added to clean 5 ml test tubes. The hops biomass suspension pH was then adjusted to pH 3.0, 4.0, 5.0, and 6.0, respectively by adding a solution of appropriate amount of dilute base (0.01 M NaOH). At each respective pH, after equilibration, 2 ml aliquots were removed and placed into clean 5 ml test tubes. Solutions of 0.1 mM lead(II) (equal to 20.7 ppm lead) were prepared from a $Pb(NO_3)_2$ salt. At each pH, 2 ml of lead(II) solution were added to the tubes containing the appropriate pH adjusted biomass, for example: the pH adjusted metal solution with pH of 2 was added to the previously pH 2 adjusted biomass pellets. This was carried out in triplicate to maintain quality assurance for each pH study. All the tubes were equilibrated by rocking for 60 min and then centrifuged for 5 min at 2500 rpm. The supernatants were transferred to clean test tubes, the final pH was recorded, and metal analysis was performed by flame atomic absorption spectroscopy. The experiment was performed in triplicate for quality assurance. This study was also repeated with three cation exchange resins containing known functional groups for comparison purposes, which are as follows: Diaion WT01S (carboxylic acid functional group), Dowex 50WX2 (sulfonic acid functional group), and Diaion WT01S (phosphate functional group), which were all obtained from Supelco corporation (Supelco Park, Bellefonte, PA 16823-0048).

2.4. Time dependency studies for lead binding

The batch laboratory time dependency studies were performed, using the procedure similar to that previously described by Gardea-Torresdey et al. [19–23]. In brief, a 250 mg sample was weighed, washed as indicated before, and then adjusted to pH 5.0. Two milliliters of 0.3 mM metal solutions (62.1 ppm) were added to 18 tubes, three for each time interval. The time intervals chosen for the time dependence studies were 5, 10, 15, 30, 60 and 90 min. After the appropriate time interval, the samples were centrifuged and the supernatants decanted into clean tubes. Final pH for all tubes were recorded and the lead concentration was determined by flame atomic absorption spectroscopy. In addition, the experiment was performed in triplicate for quality assurance.

2.5. Lead binding capacity studies

The batch laboratory methods previously described by Gardea-Torresdey et al. were used to determine the binding capacity of lead(II) to the hops biomass [19–23]. In summary, 50 mg of biomass were washed twice with 0.1 M HCl and the washing were collected and weighed to determine any biomass loss. Two milliliters aliquots of the suspension were transferred to three tubes and then centrifuged. The supernatants were saved for the metal testing. Two milliliters of 0.3 mM lead(II) solution was added to each of the tubes and biomass-free controls, which were equilibrated by rocking for 15 min. After centrifugation, the supernatants were saved for metal analysis and again 2 ml of fresh 0.3 mM lead(II) solution (62.1 ppm) was added. This was repeated until the saturation point of the hops biomass was achieved and the final pH for all supernatants was recorded. All of the sample supernatants were analyzed for remaining lead concentrations by flame atomic absorption spectroscopy. The experiment was performed in triplicate for quality assurance. This study was also repeated with three cation exchange resins containing known functional groups (carboxyl, sulfonate, and phosphate) as described previously.

2.6. Desorption of the adsorbed lead ions

The pellets from binding capacity studies with the adsorbed metal were exposed to 2 ml of either 0.1 M HCl or 0.5 M sodium citrate at pH 7.0 and equilibrated for 15 min by rocking and subsequently were centrifuged. The supernatants were collected for lead analysis and diluted to stay within the calibration range of the standard curve. The resulting pellets were once again exposed to 2 ml of either 0.1 M HCl or 0.5 M sodium citrate at pH 7.0 to ensure the removal of any remaining lead and equilibrated by rocking for 15 min (as described above). Following centrifugation, the supernatants were analyzed for lead content by using flame atomic absorption spectroscopy.

2.7. Chemical modification of hops biomass

Chemical modification of the hops biomass was achieved following previously published procedures by Gardea-Torresdey et al. [39,40] and Tiemann et al. [24]. In short, esterification of the biomass was accomplished by reacting the washed hops biomass with acidic methanol (0.1 M HCl) for 48 h at 60 °C. The biomass was then washed three times with D.I. water and lyophilized. In addition, hydrolyzation of the biomass was achieved following previously published procedures by Gardea-Torresdey et al. [39,40] and Tiemann et al. [24]. The hops biomass was reacted for 1 h with 0.1 M sodium hydroxide and then washed three times with D.I. water and then lyophilized.

2.8. Immobilization of hops biomass

The procedure used to immobilize the hops plant tissue via encapsulation within a polysilicate matrix was performed in a similar manner to that previously reported by Gardea-Torresdey et al. [19–21]. In brief, approximately 10 g of the hops biomass were weighed and washed twice with 0.1 M HCl and a third time with D.I. water, followed by centrifugation after each wash. The supernatant from the washings was collected, dried and weighed to account for the loss of biomass during washing. One hundred and twenty milliliters of 5% sulfuric acid (H_2SO_4) were mixed with enough 6% sodium silicate (Na_2SiO_3) to raise the pH of the solution to 2.0. At pH 2.0 the washed biomass was added to the solution and stirred for 10 min. An adequate amount of 6% Na_2SiO_3 was then added to slowly raise the pH of the solution to 7.0. The resulting polymer gel was washed with water and oven dried at 60 °C. The dried polymer was ground using a mortar and pestle and sieved to obtain a particle size of that between 20 and 40 mesh.

2.9. Column experiments

Approximately 6 ml of the silica-immobilized hops biomass polymer was packed in a column. A 0.1 mM solution (20.7 ppm) of lead(II) (made from a $Pb(NO_3)_2$ salt) was passed through the column at a flow rate of one-third of a bed volume (the volume of the biomass polymer in the column) per minute. Each bed volume was collected in a test tube utilizing a fraction collector. After passing the lead solutions through the column, 120 bed volumes of the effluent obtained were analyzed to determine the amount of metal bound by the column.

The metal accumulated in the silica-immobilized biomass was subsequently recovered by passing either 0.1 M HCl or 0.5 M sodium citrate at pH 7.0 through the column. Eighteen bed volumes of the resulting effluent (stripping) were collected and analyzed for lead content. In order to determine the recyclability of the immobilized hops biomass, three cycles were performed on each column. In addition, a control column, which only contained the silica polymer without any hops biomass, was used for quality control purposes.

2.10. Metal analyses

A Perkin-Elmer model 3110 atomic absorption spectrometer was used to analyze the effluents. Known standards were used to calibrate the instrument and a correlation coefficient value above 0.98 was maintained. The samples were analyzed three times and the mean values and standard deviations were determined. The samples were analyzed using an impact bead instead of a flow spoiler to increase the sensitivity of the instrument. The wavelength of 283.3 nm was used for lead analysis. The difference between the initial metal concentration and the remaining metal concentration was assumed to be bound to the biomass.

2.11. X-ray absorption spectroscopy studies

The X-ray absorption spectra were obtained from beamline 7-3 at Stanford Synchrotron Radiation Laboratory in Palo Alto California. The Pb(III) edges were collected using standard operating conditions of 3 GeV and 60–100 mA beam current. In order to reduce any dampening from thermal disorder, the lead laden biomass samples were run at approximately 20 K by using a liquid helium cryostat. Fluorescence spectra measurements of the biomass samples were acquired using a Canberra 13-element array germanium detector; however, transmission measurements were taken for the model compounds using argon filled ionization chambers. The model compounds (lead(II)acetate and lead(II)sulfide) were ground and diluted with boron nitride prior to analysis. Samples of hop leaves and stems were exposed to a solution of 1000 ppm lead(II) (prepared from a $Pb(NO_3)_2$ salt) at pH 2.0 and 5.0 to saturate all available binding sites on the hops biomass. The biomass samples and model compounds were packed into 1 mm path length aluminum sample holders with X-ray transparent Kapton tape windows and measured as solids. A Si(2 2 0) double crystal monochromator with an entrance slit of 1 mm was utilized for all the measurements. For higher harmonics rejection purposes the monochromator was detuned by 50%. All spectra were calibrated against the edge position of a lead foil internal standard. In order to improve the signal to noise ratio several scans were averaged for each XANES and EXAFS spectra.

2.12. X-ray absorption data analysis

The WINXAS software package was used to analyze the experimental EXAFS data. Following background subtraction, the EXAFS spectra were derived from the absorption spectra by subtracting a two-range spline from the total adsorption and dividing the difference by a normalizing polynomial function. Conversion of the kinetic energy of the photonelectrons to wave vector (k) values and the resulting scattering curve was weighted by k^2. Fourier transformation (FT) were obtained using a Hanning window and the interatomic

distance (*R*), number of atoms coordinated to the absorbing atom and the Debye–Waller factor were determined by least square fits from the Fourier filtered EXAFS data. The ab initio, single-scattering code FEFF (v.8.01) was used to calculate the total phase and amplitude shift functions from the structures of lead(II)acetate and lead(II)sulfide.

3. Results and discussion

Since lead contaminated waters and industrial effluents are often found at different pH, studies were performed to determine the effects of pH upon lead(II) binding by hop leaves and stems biomass. Fig. 1 shows the binding of lead(II) by the hop leaves and stems biomass as the pH is raised from 2.0 to 6.0. From Fig. 1, it can be observed that the percentage of lead bound by the hop stems and leaves increases from pH 2.0–3.0. However, following pH 3.0, the percentage of lead bound remains stable up to pH 6.0. In addition, this study was also performed with three cation exchange resins containing known functional groups (carboxyl, sulfonate and phosphate) for comparison purposes to gain further insight into the lead(II) binding mechanisms by the hops biomass. Similar pH dependent binding is observed when the trends are compared to the cation exchange resins, indicating that the hops biomass is binding lead(II) through analogous ion-exchange mechanisms. Nevertheless, the pH dependent binding of lead(II) by hop stems and leaves lies somewhere between the pH independent lead(II) binding of sulfonate and phosphate resins (strong cation exchange) and the more pH dependent carboxyl resin (weak cation exchange). This pH dependent trend implies that the mechanism of lead(II) binding by hop stems and leaves is through the deprotonation of functional groups, more similar to the carboxyl resin, and suggests that

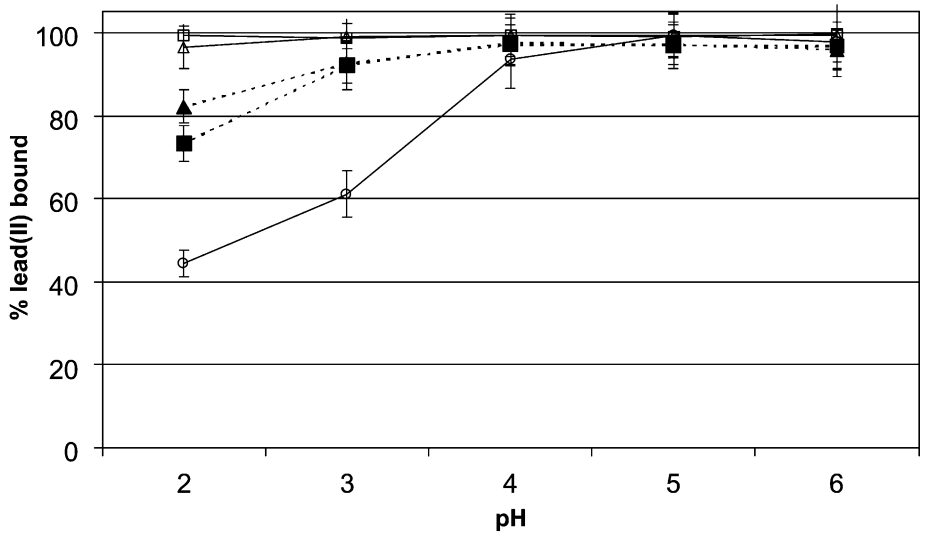

Fig. 1. Effect of pH on lead(II) binding by hops biomass compared to ion-exchange resins: hop leaves (■); hop stems (▲); sulfonic acid resin (△); phosphate resin (□); carboylic acid resin (○).

Table 1
Percentage content of carbon, nitrogen and sulfur in hop stems and leaves[a]

	Nitrogen (%)	Carbon (%)	Sulfur (%)
Hop stems	1.1 ± 0.1	36.8 ± 4.2	0.4 ± 0.1
Hop leaves	2.5 ± 0.2	32.5 ± 3.7	0.2 ± 0.1

[a] Ninety-five percent confidence interval was used to determine error.

recovery of bound lead may be achieved through reduction of pH as has been seen with other biomaterials [19–23].

In order to better understand the metal binding mechanisms of hop stems and leaves, total carbon, nitrogen and sulfur determinations for the plant tissues were performed. Table 1 shows the approximate percentages of total carbon, nitrogen and sulfur in the hop stems and leaves. The relatively low percentage of sulfur (0.4 and 0.2% for stems and leaves, respectively), in comparison to the carbon quantities, indicates that few sulfur compounds are involved in the adsorption of lead(II) by the hops biomass. However, greater percentages of nitrogen are seen in comparison to the sulfur quantities, which indicates that some nitrogen-containing compounds may be available for lead(II) adsorption. These nitrogen-containing compounds may be part of the plant cellular proteins. Student's *t*-test was conducted and showed no significant difference between hop stems and leaves as compared to the nitrogen, carbon and sulfur content.

Time dependency experiments were also performed to determine the effects of aqueous lead(II) exposures upon lead binding by the hop stems and leaves. Fig. 2 displays the time dependency for the exposure period ranging from 5 to 90 min. As seen from the figure, both stems and leaves are able to bind lead(II) from solution within the first 5 min of exposure and the level of binding is stable thereafter, indicating that the bound lead remains on the hops biomass throughout the entire reaction period via a firm

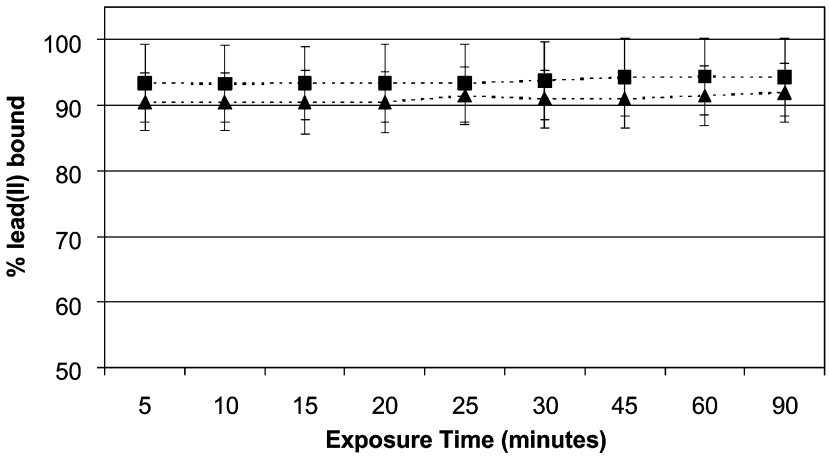

Fig. 2. Effect of exposure time on lead(II) binding by hops: hop leaves (■); hop stems (▲).

Table 2
Comparison of capacities for native and chemically modified hops biomass with ion-exchange resins for lead(II) binding and desorption[a]

Material	Milligram lead bound/gram material	Stripped (%)	
		HCl (0.1 M)	Sodium citrate (0.5 M)
Native hop stems	53.6 ± 3.1	85.2 ± 4.8	95.8 ± 9.2
Hydrolyzed hop stems	56.6 ± 2.8	84.5 ± 5.1	
Esterified hop stems	10.6 ± 2.2	25.4 ± 3.8	
Native hop leaves	74.2 ± 2.2	88.6 ± 7.9	99.1 ± 6.7
Hydrolyzed hop leaves	85.4 ± 3.5	86.2 ± 6.7	
Esterified hop leaves	10.7 ± 2.8	28.9 ± 3.5	
Sulfonic acid resin	134.5 ± 9.8	67.8 ± 3.1	
Phosphate resin	147.9 ± 3.4	77.3 ± 6.8	
Carboxylic acid resin	44.2 ± 6.3	95.7 ± 8.1	

[a] Ninety-five percent confidence interval was used to determine error.

chemical interaction. In addition, the lead(II) binding seen in Fig. 2 further indicates that the metal ions are mainly interacting with surface functional groups and not through absorption within the hop plant cells since the binding does not increase with time, which has also been seen with other biomaterials [19–23]. Therefore, the binding mechanism of the inactivated hops biomass should not be a biological one, but rather due to the surface chemistry of the natural chemical functional groups located on the surface of the hop plant tissue cell wall, similar to that observed for alfalfa and *Mucor rouxii* biomasses [24,26,40].

In order to determine the amount of lead(II) that the hops biomass is capable of adsorbing, batch binding capacity experiments were performed. Table 2 shows the binding capacity for the hop stems and leaves in milligrams of lead bound per gram of biomass. Hop leaves were shown to have a statistically significant greater capacity for lead(II) binding than the stems ($P < 0.05$), which may be due to differences in the composition and prevalence of chemical functional groups. Additional capacity experiments were performed with esterified hop stems and leaves to determine the contributions of carboxylic functional groups on the lead(II) binding. As seen in Table 2, by blocking the available carboxylic acid functional moieties by means of converting them to methyl esters, the capacity for the hops biomasses were decreased by 81% for the stems and 86% for leaves, which was similar to the results obtained with alfalfa biomass [24,26]. This decrease in lead(II) binding by the esterified stems and leaves as compared to the native hop tissues is statistically significant ($P < 0.05$) and suggests that carboxyl groups play a significant role in lead(II) binding by hop stems and leaves. Therefore, if carboxyl ligands are involved in the binding of lead(II) from aqueous solution, hydrolysis (or saponification) of the available esters on the hops biomass should produce new carboxyl groups and increase the capacity for lead(II) binding, as observed for the results obtained with sphagnum peat moss [39]. Table 2 exemplifies the enhanced binding by the hops biomass, which was nearly 6% for the stems and 15% for the leaves. However, this increase proved not to be statistically significant for the hydrolyzed biomass,

which may be due to the low content of cellulosic methyl esters in the hop plant tissues. Still these data demonstrate the differences in the composition of the stems and leaves. Both the esterified and hydrolyzed results are in agreement with other studies on metal binding which have shown that carboxyl groups are involved in metal ion binding by other biomaterials [6,18,24,39,40]. For comparison purposes, the capacity studies were repeated with the same three cation exchange resins containing known functional groups (carboxyl, sulfonate and phosphate) that were used in the pH studies (Fig. 1). As can be seen in Table 2, the capacity for lead(II) binding by hop stems and leaves was slightly higher than the weak cation exchange resin (carboxyl groups) while the strong cation exchange resins (sulfonate and phosphate groups) had statistically significant higher capacities ($P < 0.05$).

Since the pH profiles indicated reduced binding at lower pH values, exposing the lead laden biomass to dilute acid should allow for recovery of the lead from the hops biomass, which is the typical treatment for metal stripping from weak cation exchange resins [41]. Recovery of the bound lead from the hops biomass using 0.1 M HCl afforded 85% desorption for stems and 89% desorption for leaves, which are more similar to the weak cation exchange resin containing carboxyl functional groups (96% desorption) than the strong cation exchange resins which offered only 68% desorption for the sulfonate resin, and 77% desorption for the phosphate resin. Recovery of the bound lead from the hydrolyzed hops biomass using 0.1 M HCl afforded 85% desorption for stems and 86% desorption for leaves, while recovery form the esterified hops biomass afforded 25% desorption for the leaves and 29% desorption for the stems. These data along with the pH profile data indicates that the major lead(II) interaction with the hops biomass is through a mechanism more similar to the weak cation exchange resin, (carboxyl group) while a minor interaction still exists to a lesser extent as seen with the small amount of lead binding observed by the esterified hops biomass. The limited adsorption of lead(II) and low recoveries by the esterified hops biomass point to a small number of ligands that bind lead(II) via a similar mechanism to the strong cation exchange resins (sulfonate and phosphate). However, the contribution of these ligands appears to be limited, allowing us to focus more on the carboxyl functional groups than the other possible moieties. Furthermore, the desorption percentages were higher for the hops biomass and the weak cation exchange resin than that achieved for the strong cation exchange resins using dilute acid, which further supports that the mechanism of lead(II) binding by the hops biomass is via an ion-exchange process similar to the weak cation exchange resins, most likely through carboxyl groups.

If a weak cation exchange mechanism involving carboxyl ligands is responsible for the binding of lead(II) by the hops biomass, then it would stand to reason that a similar competitive mechanism could be used to further recover the bound lead from the stems and leaves. For this reason, sodium citrate was investigated to determine if it could be used as a more environmentally friendly chemical agent to recover lead from the hops biomass. Table 2 shows the results from the stripping experiments using 0.5 M sodium citrate. Lead recovery of 96% for the stems and 99% for the leaves was achieved using 0.5 M sodium citrate. These results further indicate that the binding mechanism for the majority of the ligands on the hops biomass corresponds to that of a weak cation exchange resin, similar to the carboxyl functional group.

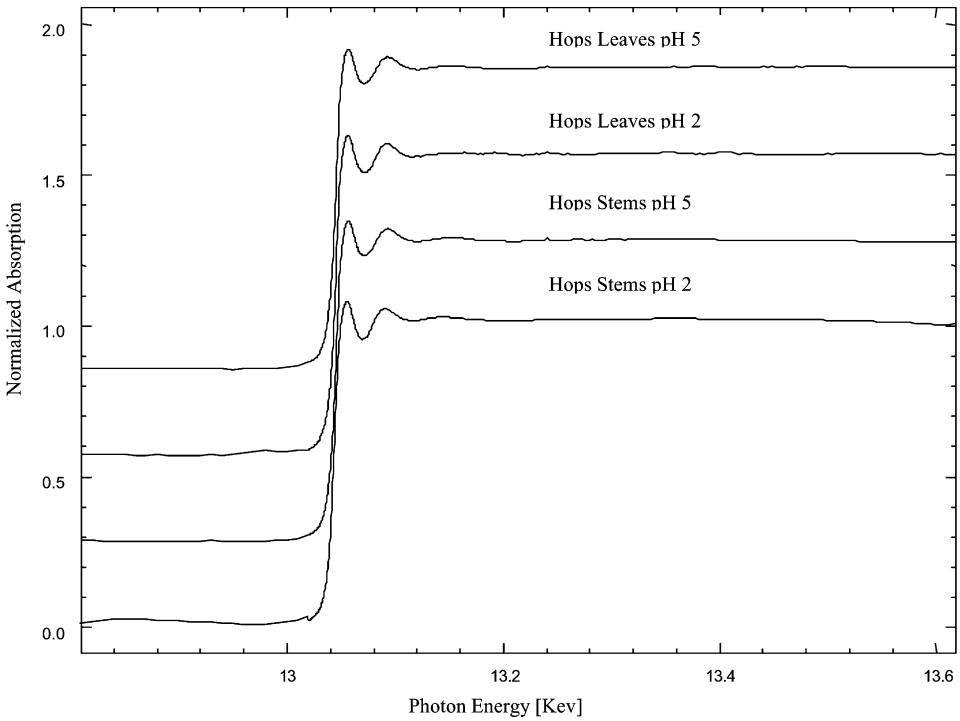

Fig. 3. X-ray absorption near-edge spectra for lead(II) bound onto hop leaves and stems biomass after being exposed to1000 ppm lead(II) at pH 2.0 and 5.0.

In order to further study, the mechanism of lead(II) binding by the hops biomass, X-ray absorption spectroscopy was performed with the lead laden biomass. XANES provides information about the absorbing metal's oxidation state as well as its geometry, while the EXAFS provides information about the absorbing metal's coordination environment [24,26]. Fig. 3 shows the XANES spectra for the hops stems and leaves following reaction with lead(II) at pH 2.0 and 5.0. The two pHs were used in the study to determine if multiple ligands may be involved in the binding of lead(II) by the hops biomass. The edge positions for the lead laden hop leaves and stems are all closely related, indicating that a similar mechanism of binding is occurring. In addition, when the edge positions for the lead laden hop leaves and stems are compared to the model compounds lead(II)sulfide and lead(II)acetate (see Fig. 4), the adsorption edge positions are within a few electron volts of each other, indicating that the lead binds to the hop stems and leaves as lead(II). However, the "tailing" region (13,040–13,100 eV) of the XANES spectra for the hops biomass strongly resemble the model compound lead(II)acetate, demonstrating that the geometry and coordination of the lead(II) bound onto the hops biomass and lead(II)acetate are very similar. The isolated FT-EXAFS for the hops stems and leaves are shown in Fig. 5. As observed in Fig. 5, all of the hop stems and leaves appear qualitatively similar with respect to the major peak observed at the approximate distance of 2 Å. However, upon closer inspection,

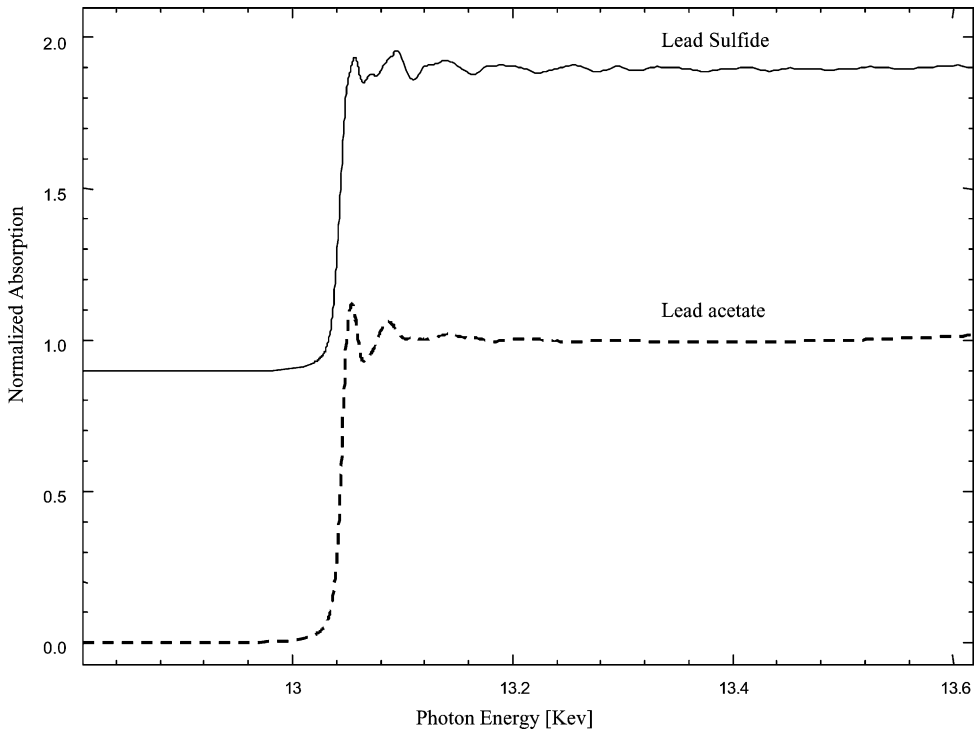

Fig. 4. X-ray absorption near-edge spectra for lead(II) model compounds. Lead(II)sulfide (—), lead(II)acetate (– – –).

a small peak is observed only in the leaves and stems exposed to lead(II) at pH 2, which is absent in the spectra of the leaves and stems exposed to lead(II) at pH 5.0. This small peak may point toward a binding mechanism involving more than two ligands with one low affinity ligand in smaller quantity at lower pH that does not have as high of an affinity for the lead(II) in solution at pH 5.0 as the primary ligand. When the EXAFS for the hop stems and leaves are compared to the model compounds lead(II)sulfide and lead(II)acetate (see Fig. 6), a strong similarity is seen between the hops biomass and lead(II)acetate containing oxygen ligands, since the major peaks are observed at the approximate distance of 2 Å, while the major peak for lead(II)sulfide is seen nearly 0.5 Å further away. These data further support that the hops biomass is binding lead(II) via oxygen containing functional groups similar to that of a weak cation exchange mechanism, which is most likely through carboxyl groups.

While the batch laboratory experiments have shown that the hops biomass is able to adsorb lead(II) from aqueous solutions, batch systems are not as practical for industrial application. Therefore, column experiments were performed to determine the binding abilities of the hop leaves under flow conditions. A silica-support matrix was utilized to help maintain flow through the column. Fig. 7A and B show the breakthrough curves for three cycles of removal and recovery of lead(II) at pH 5.0. As seen in Fig. 7A, the majority

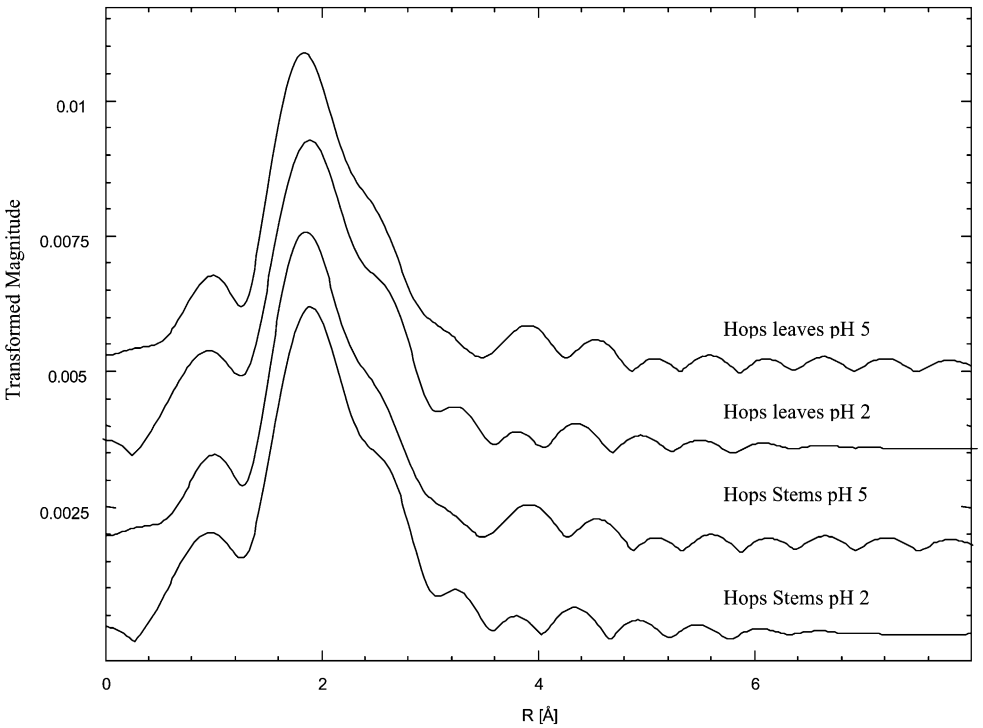

Fig. 5. Extended X-ray absorption fine structure spectra for lead(II) bound onto hop leaves and stems biomass after being exposed to 1000 ppm lead(II) at pH 2.0 and 5.0.

of the lead(II) was retained on the column as compared to the initial influent concentration. After about 60 bed volumes, a decrease in the binding was observed, due to loading of the binding sites. However, the column was not saturated until after 110 bed volumes of the 20.7 ppm solution had been passed. The total amount of lead(II) adsorbed by the silica-immobilized hops biomasses for the three cycles performed are listed in Table 3. An

Table 3
Comparison of lead(II) adsorption and recovery by silica-immobilized hops biomass[a]

Column media	Amount bound (ppm lead)			Lead recovered (%)			Stripping agent
	Cycle 1	Cycle 2	Cycle 3	Cycle 1	Cycle 2	Cycle 3	
Hop stems	1407.1	1235.9	1192.1	95.8	92.9	88.4	0.1 M HCl
Hop leaves	1912.7	1837.2	1693.5	96.1	88.0	81.0	0.1 M HCl
Hop stems	1558.3	1530.4	1471.2	98.8	99.2	99.4	0.5 M Sodium citrate
Hop leaves	2018.1	2003.8	2003.9	98.7	99.4	99.7	0.5 M Sodium citrate
Silica matrix	26.4			96.3			0.1 M HCl

[a] Influent concentration of 0.1 mM lead(II) (20.7 ppm) at pH 5.0.

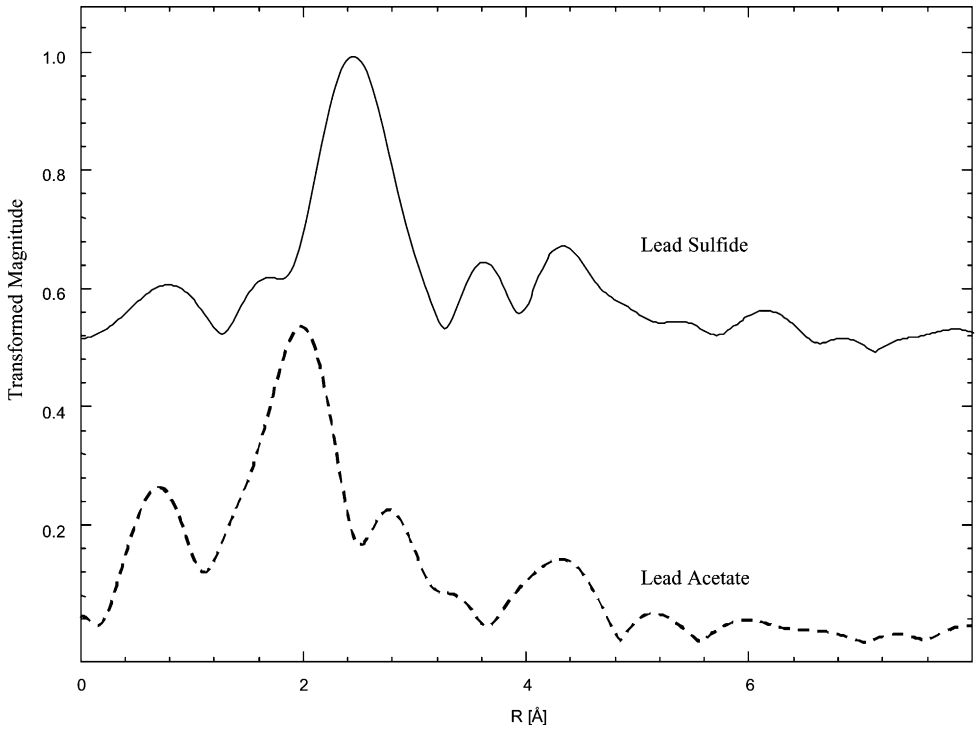

Fig. 6. Extended X-ray absorption fine structure spectra for lead(II) model compounds. Lead(II)sulfide (—), lead(II)acetate (– – –).

additional experiment was performed with a control column packed with the silica-support material (no hops biomass), which retain only 26.4 ppm of the influent solution. This small amount of lead is thought to be due to the dilution of the initial influent solution, which resulted in reduced lead concentrations in the primary bed volumes. However, the silica control column indicates that the hops biomass is responsible for the adsorption of the lead(II) from the influent solution. As with the capacity experiments, dilute acid (0.1 M HCl) was utilized to strip the bound lead from the immobilized hops biomass (Fig. 8A). As seen in Fig. 8A, the bound lead was recovered in a concentrated solution within the first 10 bed volumes. However, only 96% recovery of the bound lead was achieved (Table 3). In order to determine if the columns could be reused, the same column was again used to remove lead(II) from aqueous solution. As shown in Fig. 7A, the breakthrough occurred earlier, at approximately bed volume 45 for cycle 2 and approximately 35 for cycle 3. Also, as seen in Fig. 8A, less lead was recovered from the additional cycles. Table 3 shows that the amount of lead recovered from the hop stems and leaves decreased for cycle 2 and 3. Since sodium citrate was shown to strip nearly all of the bound lead during the batch capacity experiments, the column experiments were repeated using 0.5 M sodium citrate at pH 7.0 to strip the bound lead form the immobilized hops biomass. Fig. 7B shows the amount of lead that remained in the effluent after the solution of lead(II) at pH

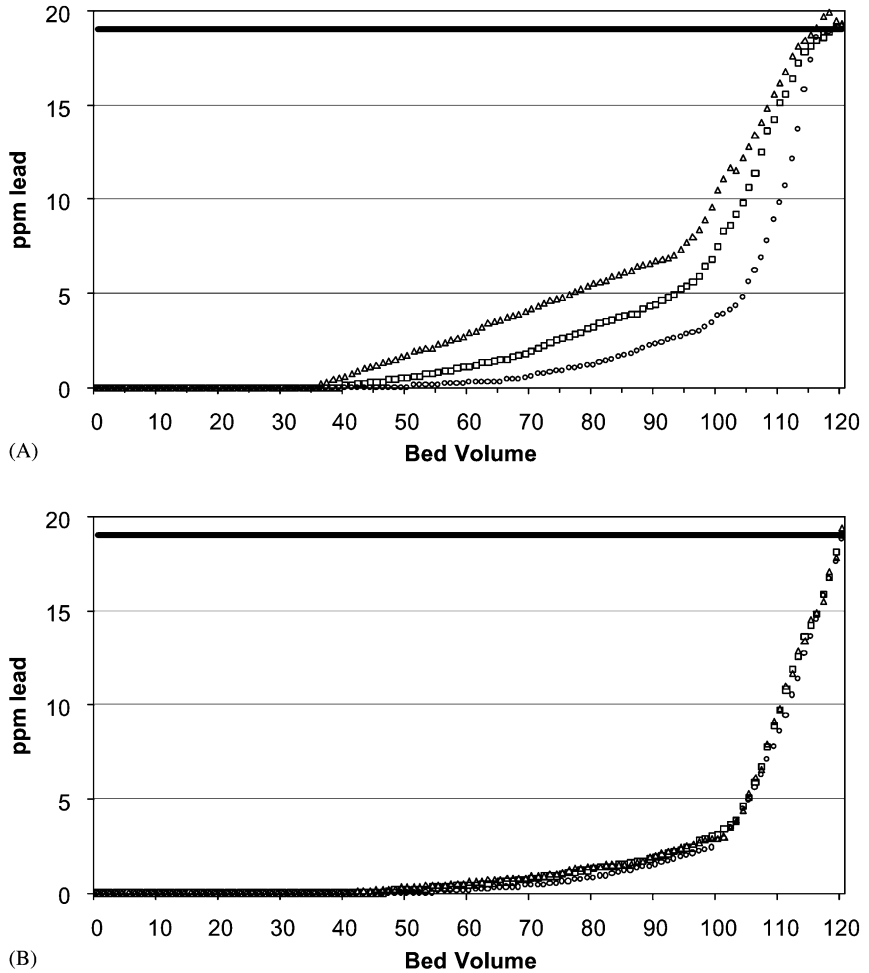

Fig. 7. Comparison of breakthrough curves for three repeated cycles of immobilized Hops biomass packed columns. (A) Column stripped with 0.1 M HCl. (B) Column stripped with 0.5 M sodium citrate. Influent concentration (—), cycle 1 (○), cycle 2 (□), cycle 3 (△).

5.0 was passed through the immobilized hops biomass. As seen in the figure, there was a dramatic increase in the reuse of the column as the breakthrough curves are almost identical for the three cycles. In addition, Fig. 8B shows the amount of lead recovered from the column per bed volume of sodium citrate passed, and again, they are nearly identical for the three cycles. Furthermore, as shown in Table 3, the sodium citrate was able to remove nearly all of the bound lead without damaging the hop leave biomass and allow for continual reuse of the hops biomass to remove lead(II) from aqueous solution. A similar trend of desorption with 0.5 M sodium citrate was observed for the hop stems (Table 3).

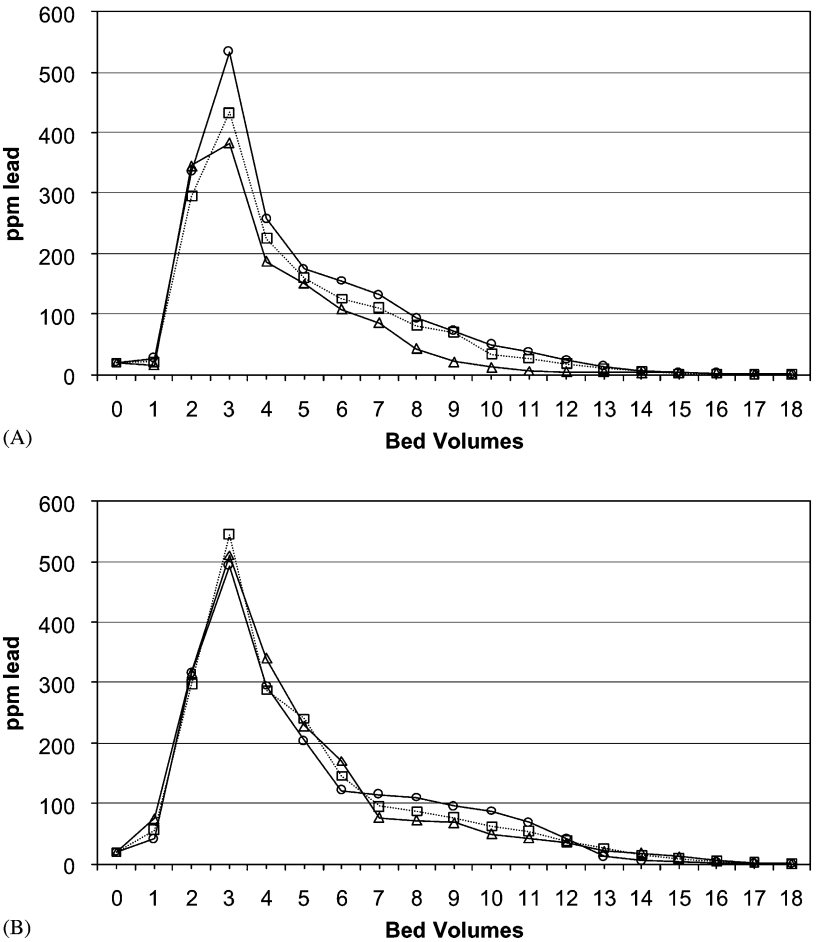

Fig. 8. Comparison of stripping data for three repeated cycles of immobilized hops biomass packed columns. (A) Column stripped with 0.1 M HCl. (B) Column stripped with 0.5 M sodium citrate: cycle 1 (○); cycle 2 (□); cycle 3 (△).

4. Conclusions

Batch laboratory experiments have allowed for the characterization of lead(II) binding by *H. lupulus* (hop) leaves and stems and have shown that the binding mechanism occurs rapidly (within 5 min) via a pH dependent mechanism, similar to that of a weak cation exchange resin containing carboxyl functional groups. In addition, these experiments indicate that the binding is occurring though surface adsorption onto the hop plant cells. Batch laboratory capacity experiments showed that the hops biomass is able to bind lead(II) in amounts comparable or better to that of other commercially available weak cation exchange resin. In addition, the hops biomass has shown a remarkable ability for recovery

of the bound lead using a more environmentally friendly chemical agent (sodium citrate) than conventional methods that use strong acids. Results from the recovery experiments were found to be comparable and even surpassed the other commercially available weak cation exchange resin used for comparison in this study. Chemical modification experiments helped to determine that carboxyl groups are involved in the binding of lead(II) by hops biomass. XANES and EXAFS studies further confirmed the binding of lead to the hops biomass as lead(II) via oxygen containing ligands. These data suggest that carboxyl groups are the major ligand involved in lead(II) adsorption by the hops biomass. Additional flow studies using silica-immobilized hop biomass were performed and have shown that the same efficiency can be achieved for at least three cycles with the same biomass using sodium citrate to recover the bound lead in a more concentrated form. This innovative technology provides a reusable material derived from agricultural waste that is not only biodegradable, but allows for the environmentally friendly removal and recovery of lead(II) from industrial waste and polluted waters.

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