

CHARACTERIZATION OF CHROMIUM(VI) BIOREDUCTION AND CHROMIUM(III) BINDING TO ALFALFA BIOMASS

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ABSTRACT

Chromium(VI) is a common waste product generated from industrial processes such as electroplating, wood preservation, and metal finishing, and is highly toxic as compared to chromium(III). Current disposal and treatment methods, such as ion exchange resins, are costly and pose a great health risk to the public. A more environmentally sound and cost-effective method of phytoremediation using alfalfa biomass may be a plausible solution. Previous studies have shown alfalfa to have a high affinity for chromium(III) at pH 5 and 6 under single-metal and mixed-metal conditions. Flow studies performed using silica-immobilized alfalfa biomass were conducted and X-ray microfluorescence (XRMF) was used to show conclusive evidence of chromium(III) binding. Studies performed using X-ray absorption near edge structure (XANES) provided direct evidence that alfalfa biomass reduces chromium(VI) to chromium(III). Time and temperature studies conducted at pH 2 and 5 were also utilized to study the reduction process of chromium(VI) to chromium(III). Temperature experiments showed that chromium(III) binding increased as the temperature increased under single- and mixed-metal conditions. However, chromium(VI) binding does not follow the same temperature-dependent trend. This information will lead to the development of an environmentally friendly and cost-effective phytoremediation system using alfalfa biomass.

Key words: XANES, EXAFS, XRMF, alfalfa, bioadsorption, phytoremediation, chromium

INTRODUCTION

Hexavalent and trivalent chromium gain access to ground and surface waters through industrial processes such as electroplating, metal finishing, wood preservation, and tanning. Both valences of chromium are potentially harmful at high concentrations, but hexavalent chromium poses a greater health risk to the public. Hexavalent chromium is considered a primary contaminant due to its ability to gain access to groundwater and soil, where it has detrimental effects on humans as well as animals (Kortenkamp et al., 1996). Chromium(VI) has been found to be a cancer-causing agent and poses other health risks such as liver damage, dermatitis, and gastrointestinal ulcers (Carson et al., 1986; Brown and Kodama, 1987). On the other hand, in median concentrations, chromium(III) is a more stable, less toxic, and listed as an essential element (in trace concentrations) to provide good health (Katz and Salem, 1994).

Conventional treatment methods utilized in electroplating and metal finishing plants involve a lengthy process, have high disposal and chemical costs, and do not completely reduce chromium(VI) (Srivastava et al., 1989). Contaminated waters containing low concentrations of chromium(VI) are typically treated with ion exchange resins, but this method is also costly due to the high price of the resin.

In view of these treatment shortcomings, a variety of inexpensive biomasses have been studied for their ability to uptake chromium(VI) and chromium(III). Among these biomasses are seaweed,

water hyacinth, sawdust, algae, and alfalfa (Darnall et al., 1986; Kratochvil et al., 1998; Lytle et al., 1998; Singh et al., 1992; Tiemann et al., 1998; Tiemann et al., 1999). Further studies have been conducted on the ability of the biomasses to reduce toxic chromium(VI) to less toxic chromium(III). The reduction of chromium(VI) can be accomplished abiotically by redox reactions with aqueous ions, electron transfer at mineral surfaces, or reduction by lyophilized plant tissue (Lytle et al., 1998; Wittbrodt and Palmer, 1996).

Also, most treatment processes must ensure removal of both chromium(VI) and chromium(III), but current biosorption studies only focus on the removal of a single form of chromium. The characteristics under which biomasses bind either form of chromium are vastly different and very little research has been done in order to relate these two mechanisms.

Previous studies have shown that ground alfalfa biomass binds chromium(III) by way of carboxyl ligands (Tiemann et al., 1999). Alfalfa may also possess a supply of electron donors for chromium(VI) reduction. With this in mind, alfalfa may have the ability to uptake both forms of chromium. The objective of this study is to determine the mechanism of chromium(VI) binding and/or bioreduction by alfalfa biomass. The study employs the use of X-ray Absorption Spectroscopy (XAS), X-ray microfluorescence (XRMF), and batch experiments to ascertain the ability of the alfalfa biomass to bioreduce chromium(VI) to chromium(III).

MATERIALS AND METHODS

Alfalfa Collection. The alfalfa plants were collected from field studies conducted at New Mexico State University near Las Cruces, New Mexico. The alfalfa plants were removed from the soil and washed with water to remove dirt and debris. The root material was separated from the shoot material which contains the stems and leaves. The samples were oven dried for a week at 90° C and ground with a Wiley mill to pass through a 100-mesh sieve.

pH Profile Studies for Chromium Binding. The protocol for this experiment was carried out according to the method previously outlined by Gardea-Torresdey et al. (1996). A metal solution containing 0.1 mM of chromium(III) was prepared from $\text{Cr}(\text{NO}_3)_3$ and a 0.1 mM solution of chromium(VI) was prepared from $\text{K}_2\text{Cr}_2\text{O}_7$. The solutions were adjusted to pH 2, 3, 4, 5, and 6, and reacted with their respective pH biomass pellet for one hour. This experiment was performed in triplicate for statistical purposes and to maintain quality control. The supernatants were collected and final pHs were recorded. Metal analysis was performed by flame atomic absorption spectroscopy.

Time Dependence Studies for Chromium Binding. The time-dependence procedure followed was similar to that previously reported (Tiemann et al., 1998). Two 0.3 mM metal solutions were prepared, one of chromium(III) and the other chromium(VI) using the same chromium salts described earlier. The chromium(III) solution and corresponding biomass pellets were adjusted to pH 5.0 and reacted for time periods of 5, 10, 15, 20, 25, 30, 45, 60, 90, 360, 720, 1440, and

2880 minutes. The experiment was repeated again with the chromium(VI) solution and corresponding biomass pellets adjusted to pH 2.0 and reacted for the same time intervals. Both of these experiments were performed in triplicate for statistical purposes and to maintain quality control. The supernatants were collected and the final pH of each sample was recorded. Metal analysis was performed by flame atomic absorption spectroscopy.

Chromium-Binding Capacity Studies. The method followed for the metal-binding capacity experiments which were used to determine the capacities and binding affinities of the individual metal ions for the alfalfa biomass was similar to that previously reported (Tiemann et al., 1998). Two 0.3 mM metal solutions were prepared, one with chromium(III) at pH 5.0 and the other with chromium(VI) at pH 2.0 as described earlier. The alfalfa biomass was then reacted with its respective solution for 15 minutes. The samples were centrifuged and the supernatants were collected. This process was repeated nine times or until the biomass became saturated. This experiment was performed in triplicate for statistical purposes and to maintain quality control. The final pH of the supernatants was recorded and metal analysis was performed by flame atomic absorption spectroscopy.

Desorption of Adsorbed Chromium. The biomass pellets from the capacity studies were reacted with 0.1M hydrochloric acid (HCl) for 15 minutes in order to remove the bound metals from the biomass as previously described (Tiemann et al., 1998). The supernatants were collected and this process of acid treatment was repeated twice. The final pH of the supernatants was recorded and metal analysis was performed using flame atomic absorption spectroscopy.

Temperature Dependence Studies for Chromium Binding. A 250-mg sample of alfalfa biomass was washed twice with 0.1 M HCl and twice with DI water in order to remove any soluble material that may interact with the metal ions. The washings were collected, dried, and weighed to account for any biomass weight loss. The biomass was resuspended in 50 ml of deionized (DI) water to make a 5-mg/ml solution mixture. The pH of the mixture was adjusted for chromium(III) experimentation. Another mixture was prepared the same way with the alfalfa biomass and the pH was adjusted to 2.0 for chromium(VI) experimentation. Three clean plastic 5 ml test tubes were used for each form of chromium and labeled for each temperature: 4°C, 25°C, and 55°C. Then 2 ml of the biomass mixture was transferred to each respective tube. Each tube was centrifuged at 3000 rpm for five minutes and the supernatants were discarded. The tubes labeled 4°C were put in a refrigerator; the 25°C were left out on a lab bench; and the 55°C were put in an oven so they could reach their respective temperatures. Two 0.3 mM chromium solutions were made as earlier described and the pH was adjusted to pH 5.0 for Cr(III) and pH 2.0 for Cr(VI). After adjustment of the pH, 2 ml of metal solution was put in each test tube. The test tubes were equilibrated on a rocker at their corresponding temperatures. Two control solutions, one for chromium(III) and one for chromium(VI), were equilibrated at each temperature. After a one-hour reaction period, the

samples were centrifuged and the supernatants were removed from their pellets and saved for analysis. The final pH of each supernatant was recorded and chromium analysis was performed by flame atomic absorption spectroscopy.

The apparent enthalpies were calculated using Van't Hoff's equation as seen below:

$$\ln\left(\frac{K_2}{K_1}\right) = \frac{(\Delta H^\circ (T_2 - T_1))}{RT_2T_1}$$

The equilibrium constant values (K) were replaced by distribution ratio values (D). These distribution ratios are calculated from ion exchange calculations. They are the ratio of the concentration of metal ion adsorbed to the concentration of metal ions in the solution at equilibrium (Green and Darnall, 1988). Even though distribution ratios are not true thermodynamic equilibrium constants, their use is justified by the complexity of the alfalfa tissues. The heats of formation calculated are merely relative values that enable determination of whether the process was endothermic or exothermic.

Chromium Analyses. The chromium analysis in each experiment was performed using a Perkin Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. A calibration curve was obtained using known chromium standards and a correlation coefficient of 0.98 or greater was obtained for the analyses. All samples were read three times and the mean value and relative standard deviation were calculated. The wavelength used for chromium analysis was 358.2 nm. Instrument response was checked using a known chromium standard.

X-Ray Microfluorescence Analysis (XRMF). Samples of alfalfa control and alfalfa biomass loaded with chromium(III) and chromium(VI) taken from chromium-binding capacity studies were analyzed using X-ray microfluorescence. The samples were also treated with 0.1 M HCl and analyzed with X-ray microfluorescence for chromium. The samples were set on an X-ray polyester thin film support and analyzed with a Kevex Omicron X-ray Microfluorescence spectrometer. A 100 μm collimator with an energy-dispersive solid state detector was used. The operating conditions were as follows: energy molybdenum tube at 40keV and up to five minutes counting time.

X-Ray Absorption Spectroscopy (XAS). Samples of alfalfa biomass were washed twice with 0.1 M HCl and twice with DI water. The samples were then reacted with 1000 ppm solutions of chromium(III) and chromium(VI) for one hour at pH 5 and pH 2, respectively. After the reaction period, the samples were centrifuged at 3000 rpm for five minutes. The supernatants were removed and placed into clean tubes. The samples were then washed three times with DI water to remove any unbound chromium. This procedure was repeated for the weak cation exchange resin samples (Diaion WT01s from Supelco).

X-ray absorption measurements were made at beam line SBO7-3(1.8T Wiggler Field) using a 13-element germanium array detector at the Stanford Synchrotron Radiation Laboratory(SSRL).

The samples were maintained at a temperature of approximately 10K using an Oxford Instruments CF 204 liquid helium flow cryostat and 5x15x1 mm cells with tape windows. The chromium k-edge was used to probe the chemical form of the metal. Model compounds of chromium nitrate and potassium dichromate were analyzed to be compared with the samples. The absolute energy positions were calibrated against the inflection point energy of a chromium foil. The EXAFSPak analysis package was used to analyze the data with standard methods. The E0 values were determined from the absorption-edge inflection point. Nonlinear fits based on the general Extended X-ray Absorption Fine Structure (EXAFS) equation were used to quantitatively compare unknowns to standards.

RESULTS AND DISCUSSION

Figure 1 displays the percent of chromium bound to the biomass as a function of pH from the chromium solutions. As can be observed, chromium(III) binding increases as pH increases from 2 to 6. The opposite trend can be seen for chromium(VI), where optimal binding occurs at pH 2. This opposing trend shows that each oxidation state of chromium binds to different ligands on the alfalfa biomass. Tiemann et al. have shown that chromium(III) binds by way of electrostatic interactions with the carboxyl ligand at a higher pH (1999). However, chromium(VI) exists as an oxoanion in solution and does not bind through carboxyl ligands; but by other ligands, that may be present on the surface of the alfalfa biomass.

Figure 2 represents the percent chromium bound by the alfalfa biomass for different reaction periods at pH 5 for chromium(III) and at pH 2 for chromium(VI). The binding is rapid for both oxidation states of chromium but remains constant throughout a 2880 minute period only for chromium(III). There is an increase in binding for chromium(VI) over 2880 minutes that may be associated with the reduction of chromium(VI) to chromium(III) over time.

Table 1 displays the metal-binding capacities for chromium(III) and chromium(VI) in μg of chromium per gram of alfalfa biomass. As can be seen in the table, alfalfa has a higher capacity for chromium(III) at 16.24 μg of chromium(III) per g of alfalfa biomass as compared to 2.56 μg of chromium(VI) per g of alfalfa biomass for chromium(VI). The large deviation between chromium(III) and chromium(VI) binding capacities is probably due to the fact the chromium(VI) exists as an oxoanion and does not predominantly bind to alfalfa by carboxyl ligands. Table 1 also displays the recovery of chromium(III) and chromium(VI) by treatment with 0.1M HCl. In both cases, the recovery was below 50%, which may indicate that both hexavalent and trivalent chromium are at the same oxidation state, and this would explain why dilute acid is not strong enough to overcome this interaction.

Figure 3 depicts the percent chromium bound with respect to temperature (Celsius) at pH 5 for chromium(III) and pH 2 for chromium(VI). The alfalfa biomass binds more chromium(III) than chromium(VI) in both temperature ranges of 4-25 $^{\circ}$ C and 25-55 $^{\circ}$ C. Also seen in Figure 3,

chromium(III) increases in binding in the higher temperature range while chromium(VI) binding remains relatively constant as the temperature increases. The apparent enthalpies for chromium(III) and chromium(VI) binding were calculated and are shown in Table 2. In general, formations between metal ions and carboxyl ligands are characterized by a small positive enthalpy, while formations of metal ions between sulfhydryl and amino ligands are characterized by a large negative enthalpy (Green and Darnall, 1988). In both temperature ranges, chromium(III) has a positive enthalpy, which indicates binding by carboxyl ligands. At the higher temperature range (25-55°C), the enthalpy becomes more endothermic which leads to the increase in Cr(III) binding. However, chromium(VI) displays a negative enthalpy for both temperature ranges which suggests the involvement of other ligands besides carboxyl groups. There is an increase in enthalpy from the 4-25°C range to the 25-55°C range which may indicate that reduction of Cr(VI) to Cr(III) may be occurring at a higher rate. The reduced chromium can then bind to carboxyl groups causing the enthalpy in the 25-55°C range to be more positive than that of the 4-25°C range. This process has also been observed in other biomasses (Greene and Darnall, 1988; Singh et al., 1992).

In order to corroborate with the batch studies, X-ray microfluorescence(XRMF) was conducted on alfalfa samples reacted with chromium(III) and chromium(VI) at their respective optimal binding pH. Both samples displayed high chromium peaks on the XRMF spectra (data not shown). This provides further evidence that chromium binds to the alfalfa. XRMF was also conducted on samples of alfalfa biomass with chromium(III) and chromium(VI) after desorption with 0.1M HCl. The chromium peaks can still be seen in both XRMF spectra (data not shown), which shows that not all of the chromium was removed during the desorption process. This data coincides with the binding-capacity experiment explained earlier.

X-ray absorption spectroscopy techniques were utilized to examine the reduction process of Cr(VI). X-ray Absorption Near Edge Structure or XANES was used to determine the oxidation state of the chromium bound on the alfalfa biomass. Extended X-ray Absorption Fine Structure or EXAFS was performed to ascertain which ligands chromium(III) and chromium(VI) were binding to on the surface of the alfalfa biomass. A weak cation exchange resin that contains carboxyl ligands reacted with chromium(III) was used in comparison with the alfalfa samples to mimic a situation where only carboxyl ligands were involved. The model compounds, chromium nitrate and potassium dichromate, were utilized to show the differences associated with both oxidation states of chromium.

Figure 4 is a XANES spectra of the model compounds chromium nitrate and potassium dichromate. The XANES of potassium dichromate gives a pre-edge peak at about 5990 eV, which is distinct only to chromium(VI). The XANES for chromium(III) does not contain a pre-edge which makes it easy to distinguish between both oxidation states. Figure 5 is a XANES of the model compounds and a sample of alfalfa biomass reacted with a 1000 ppm Cr(III) solution at pH 5. From Figure 5, it can be seen that Cr(III) is bound to the alfalfa biomass as Cr(III). Figure 6 is a

XANES of the model compounds and a sample of alfalfa biomass reacted with a 1000 ppm Cr(VI) solution at pH 2. The spectrum for the alfalfa sample follows that of Cr(III) which is confirmed by the absence of a pre-edge absorption peak. This means that the Cr(VI) is somehow reduced to Cr(III) by the alfalfa biomass. Figure 7 depicts the EXAFS of chromium(III) reacted with the resin and chromium(III) and chromium(VI) reacted with the alfalfa biomass. The EXAFS for each sample contain a distinct peak at about 2Å which is characteristic of coordination with an oxygen atom. This oxygen atom is quite possibly the carboxyl ligand. The chromium(III) reacted with the weak cation exchange resin, which contains carboxyl ligands, clearly displays a peak at about 2Å. This indicates that chromium(III) may bind to alfalfa by way of carboxyl ligands.

CONCLUSIONS

The pH profile studies show that different ligands are involved in the binding of Cr(III) and Cr(VI). Chromium(III) has an optimal binding pH of 5.0 which designates that carboxyl groups play a main role in the binding of Cr(III) to alfalfa biomass. Chromium(VI) has an optimal binding pH of 2.0 suggesting the involvement of other chemical groups on the surface of the alfalfa biomass. Time-dependent studies show an increase in binding over time for Cr(VI) because Cr(VI) is being reduced to Cr(III). Temperature-dependence studies reveal that the bioreduction increases at higher temperatures thus increasing Cr(III) binding by way of carboxyl ligands. XAS studies confirm that Cr(VI) is reduced to Cr(III) and subsequently the reduced chromium is bound via an oxygen-containing ligand, most likely a carboxyl group. The mechanism of the bioreduction process is still not fully understood. It is not known when the reduction process occurs, or which groups are responsible for the bioreduction process. Further studies are needed to determine the bioreduction mechanism(s) to develop an innovative system to remediate chromium contaminants from polluted waters.

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Table 1. μg of chromium bound by each gram of alfalfa biomass and percent chromium recovered after addition of 0.1 M HCl.

Chromium Adsorption Capacities for Alfalfa Biomass		
Form of Chromium	μg chromium/ g alfalfa biomass	% Chromium Recovery
Cr(III)	16.24	31
Cr(VI)	2.56	47

Table 2. Apparent enthalpies for binding of Cr(III) at pH 5 and binding of Cr(VI) at pH 2 to alfalfa biomass.

Apparent Enthalpies		
Temperature Range	Cr(III) at pH 5	Cr(VI) at pH 2
4-25°C	6.55	-23.5
25-55°C	16.9	-0.95

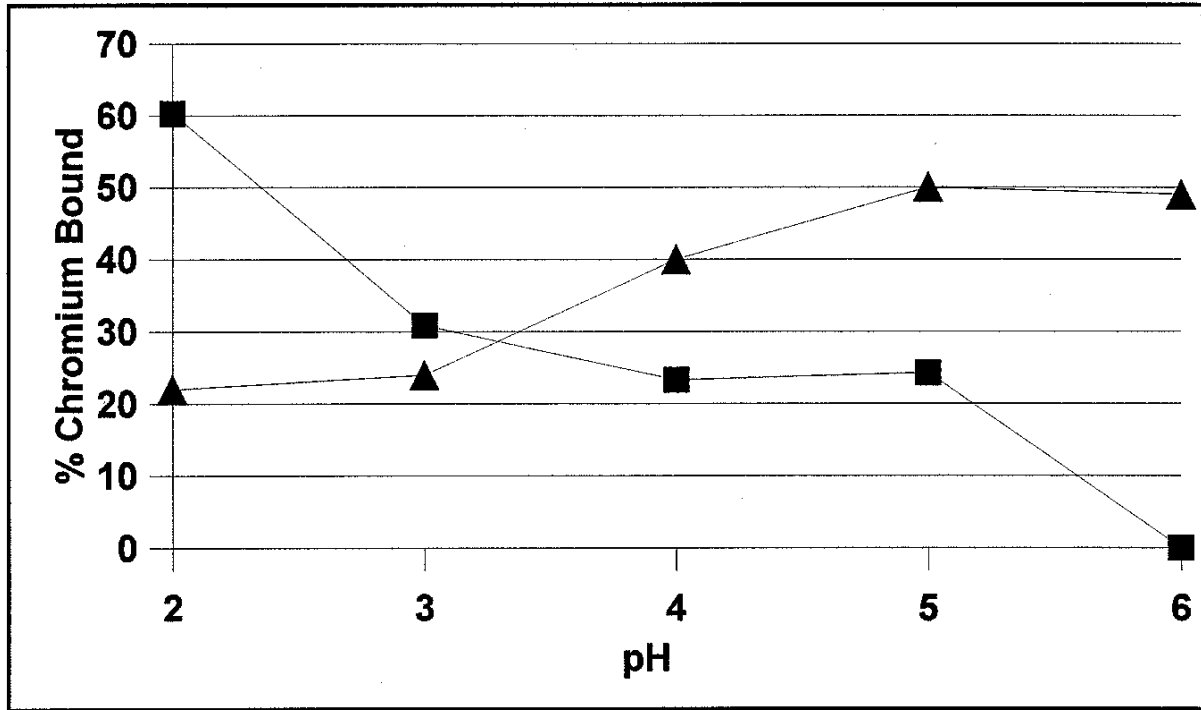


Figure 1. Percent of chromium bound by alfalfa biomass after one hour of equilibration with 0.1 mM Cr(VI) and Cr(III) solutions at various pHs: Cr(VI) (■), Cr(III) (s).

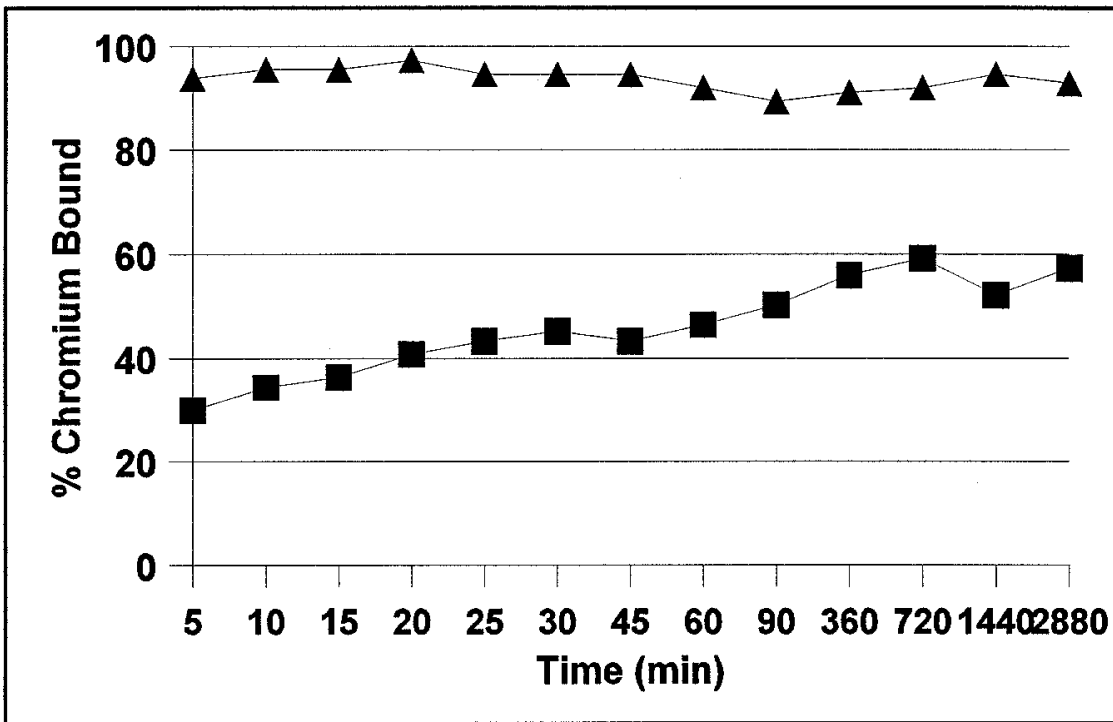


Figure 2. Percent of chromium bound by alfalfa biomass at different time intervals with a 0.3 mM Cr(VI) solution at pH 2 and a 0.3 mM Cr(III) solution at pH 5: Cr(VI) (■), Cr(III) (s).

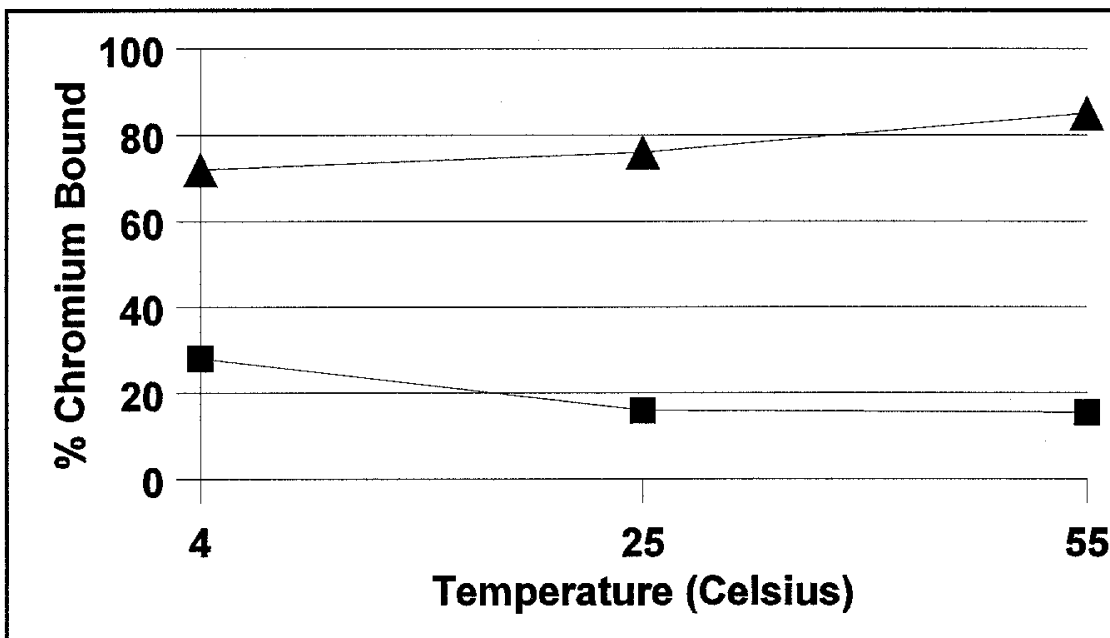


Figure 3. Percent chromium bound by the alfalfa biomass at 4, 25, and 55°C with a 0.3 mM Cr(VI) solution at pH 2 and a 0.3 mM Cr(III) solution at pH 5: Cr(VI) (■), Cr(III) (s).

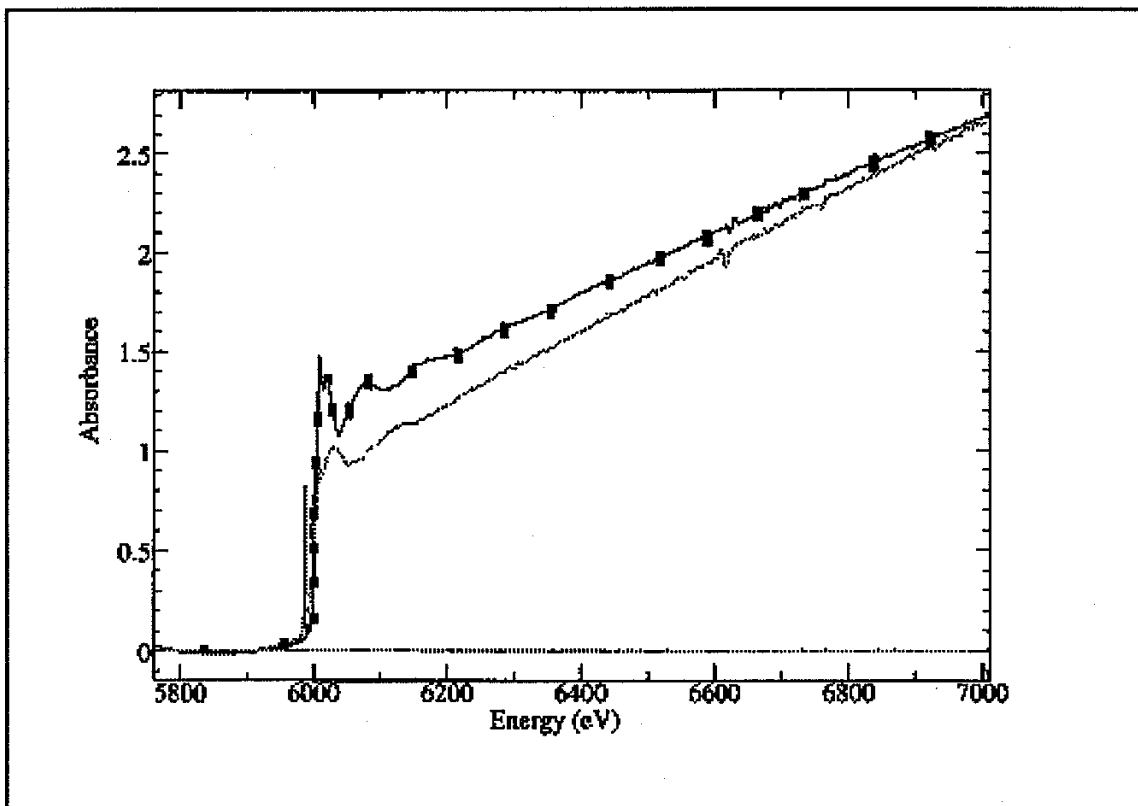


Figure 4. XANES spectra of the model compounds: Cr₃NO₃ (■), K₂Cr₂O₇ (-).

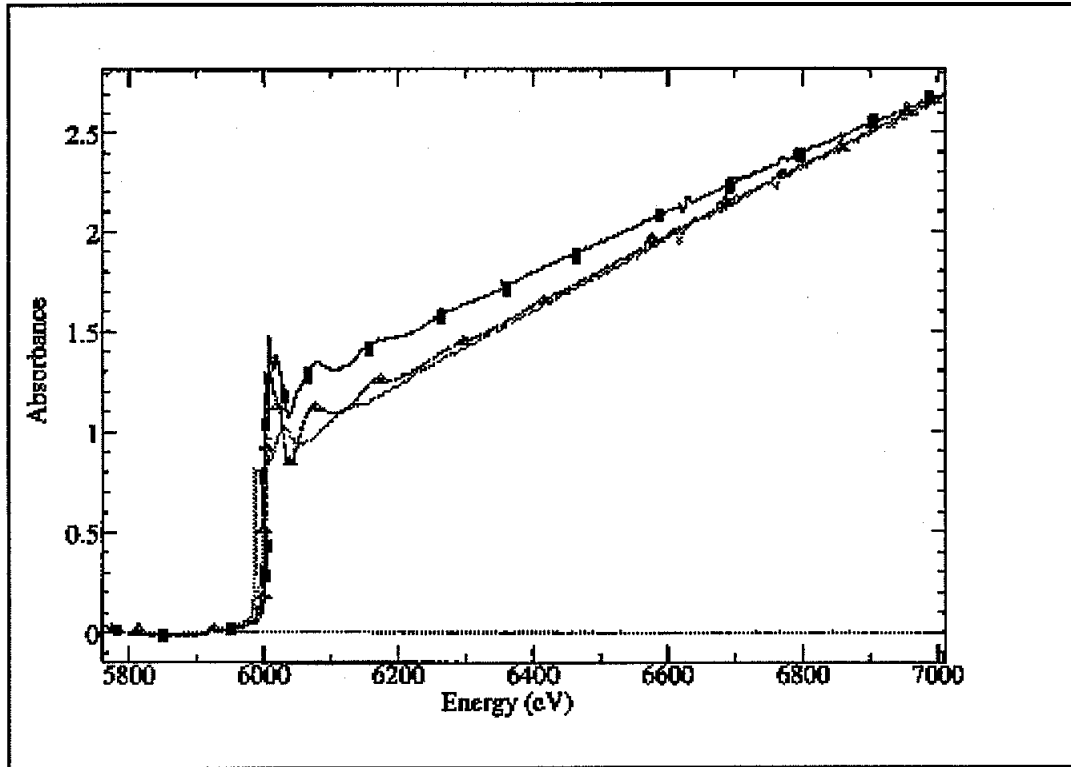


Figure 5. XANES spectra of model compounds of chromium and alfalfa biomass reacted with a 1000 ppm Cr(III) solution at pH 5, (s) : Cr₃NO₃ (■), K₂Cr₂O₇ (-).

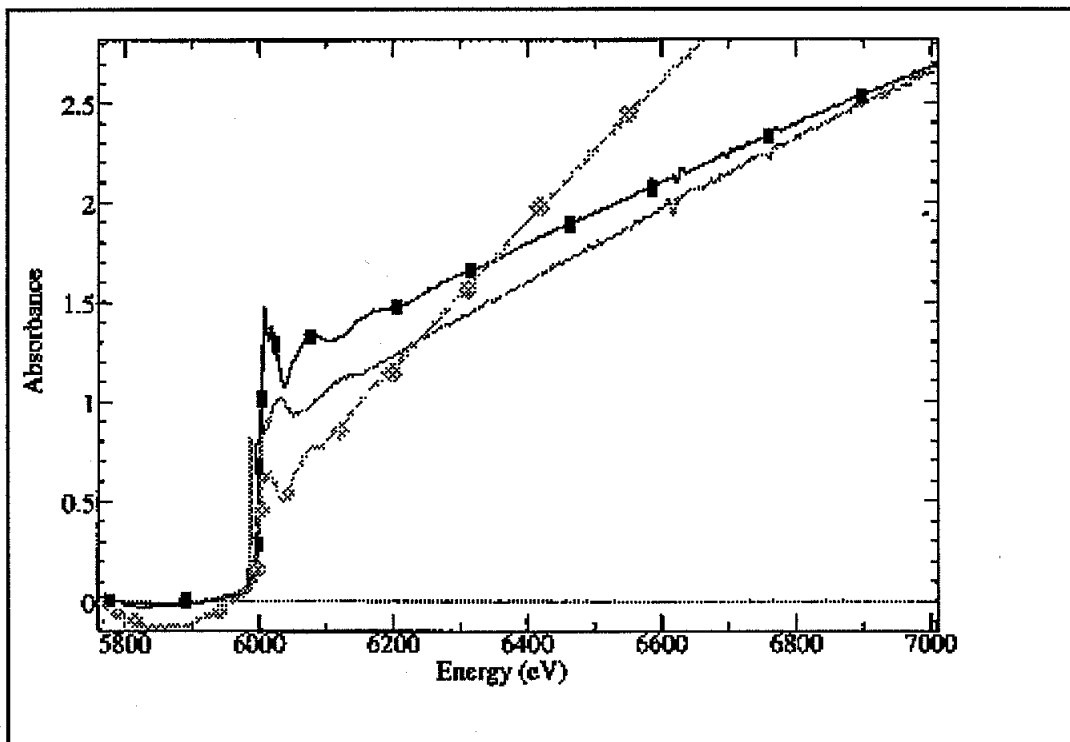


Figure 6. XANES spectra of model compounds of chromium and alfalfa biomass reacted with a 1000 ppm Cr(VI) solution at pH 2, (●) : Cr₃NO₃ (■), K₂Cr₂O₇ (-).

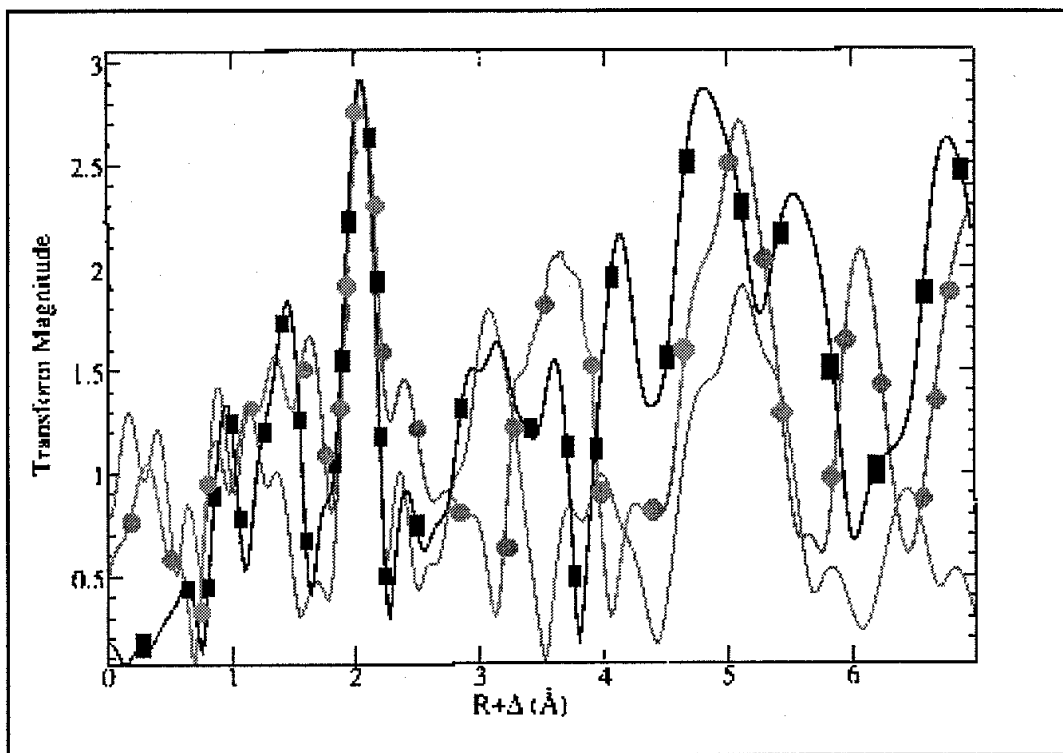


Figure 7. EXAFS of alfalfa biomass reacted with a 1000 ppm Cr(III) solution at pH 5, (■), alfalfa biomass reacted with a 1000 ppm Cr(VI) solution at pH 2, (●), and weak cation exchange resin reacted with a 1000 ppm Cr(III) solution at pH 5, (-).