ELECTROCHEMICAL STUDIES OF Cu(II) BINDING TO ALFALFA BIOMASS

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ABSTRACT

We have performed several studies to determine the electrochemical properties of Cu(II) binding by alfalfa biomass using carbon paste alfalfa-modified electrodes. Experimental conditions such as alfalfa-carbon paste concentrations, solution pH, and metal preconcentration times were determined. Results of the electrochemical studies have shown that an increase in response was observed by increasing the concentration of alfalfa biomass with a maximum response at 30% (w/w). In addition, the response was found to escalate as the pH of the Cu(II) solutions were raised from 2-6, which parallels the trends observed from previously performed batch experiments. This information will aid in the elucidation of the mechanisms for metal ion binding by alfalfa biomass in order to develop a better method to clean heavy metal-contaminated waters.

***Key words**: modified electrodes, alfalfa, bioaccumulation, metal binding, copper*

INTRODUCTION

For more than a decade, researchers have been looking for cheaper, more effective methods to remediate heavy metal-contaminated waters and reduce the growing public health risk (Runnells et al., 1992). Traditionally, methodologies such as ion-exchange and reverse osmosis are employed to remove contaminants from polluted waters. Due to the high cost associated with these methods, many polluted areas prove to be cost-prohibitive to clean. However, the use of biological systems may alleviate some of these concerns. Biological systems have proven to be quite effective at removing metal ions from contaminated solutions in a low-cost and environmentally friendly manner (Atlas, 1995).

Many researchers have investigated the use of live biological systems as a new alternative for the accumulation of heavy metal contaminants from waste waters (Cervantes and Gutierrez-Corona, 1994; Zang and Majidi, 1994; Bender et al., 1994; Nagendra et al., 1993; Rome and Gadd, 1991). Nevertheless, research performed with nonliving algal and peat moss biomasses have shown a great potential for removal and recovery of heavy metal ions (Ramelow et al., 1993; Viraraghavan et al., 1993; Lujan et al., 1994). In addition, tissues from nonliving plant materials have shown several advantages for remediation, which include the nondestructive effects from high levels of contamination and low cost (Gardea-Torresdey et al., 1996a; 1996b; 1999a; 1999b). However, due to a lack in the understanding of the fundamental mechanism underlying the metal-biomass interaction, the advancement of bioremediation research has been limited. Few studies have been performed to determine the actual metal binding mechanisms

of the biological materials for the purpose of bioremediation (Gardea-Torresdey et al., 1996c; 1996d; 1997; 1998;Tiemann et al., 1999; 2000).

Previously performed experiments with nonliving biomass of *Medicago sativa* (alfalfa) have shown that it is able to bind appreciable amounts of cadmium(II), chromium(III), copper(II), gold(III), lead(II), nickel(II), and zinc(II) (Gardea-Torresdey et al., 1996c; 1996d; 1997; 1998;Tiemann et al., 1999; 2000). In addition, these studies have shown that alfalfa biomass can selectively bind certain metal ions in mixed-metal solutions, even under the influence of hard waters which typically foul commercial ion-exchange resins (Gardea-Torresdey et al., 1999d). In spite of the biomass's metal-binding abilities, little is known about the actual chemical mechanisms involved in the selective binding and recovery of these metal ions by alfalfa biomass.

The objective of this work is to determine the properties of Cu(II) binding by alfalfa biomass using electrochemical techniques. Carbon paste alfalfa-modified electrodes were employed in this study to perform the voltammetric analysis in order to gain a better insight into the metal-biomass interactions. Experimental conditions such as optimal alfalfa-carbon paste concentrations, solution pH, and metal preconcentration times were determined. The information gathered by this study will help in the understanding of Cu(II) binding by alfalfa biomass and aid in determining better methods to recover metal ions from heavy metal-contaminated solutions.

MATERIALS AND METHODS

Biomass Preparation

The alfalfa biomass used for this study was of the Malone variety and had been selected for its metal-binding abilities as determined from previous studies (Gardea-Torresdey et al., 1996a; 1996b; 1996d; 1998;1999a; 1999b;1999c;Tiemann et al., 1999; 2000). Only the shoot materials (stems and leaves) were used in this study. The biomass was removed from the field and washed clean with water. Following a drying cycle of one week at 90°C, the biomass was ground by a Wiley mill to pass a 100-mesh sieve. Before any experiments were performed, the ground alfalfa biomass was washed twice with 0.01M hydrochloric acid (HCl) using methods previously described in order to remove any metals that might be on the biomass prior to experimental metal ion exposure (Gardea-Torresdey et al., 1996a; 1996b; 1996d; 1998;1999a; 1999b;1999c;Tiemann et al., 1999; 2000). The acid-washed biomass was then washed twice again with deionized (DI) water to remove the acid prior to any further experimentation.

Electrode Preparation

Chemically modified carbon paste electrodes (CME) were prepared by hand mixing washed alfalfa biomass with 0.375g of mineral oil and varying amounts of graphite powder 60% (w/w) to 30% (w/w) depending on percent of biomass) using a mortar and pestle. The amount of biomass used in the graphite oil mixture varied in the study from 10% (w/w) to 40%(w/w) to obtain a total of 1.25g of bio-

mass, graphite, and oil in the paste. The resulting carbon-biomass paste was then packed into the end of a glass tube (2mm i.d and 2.5mm o.d.) and in contact with a copper wire protruding from the opposite end.

Electro-Analytical Procedure

Three 20-mL cells were used in this study: the preconcentration cell containing the copper(II) solution to test the CME; the measurement cell containing the supporting electrolyte (0.05M sodium acetate at pH 5.0); and the cleaning cell containing 0.1M HCl. In order to obtain the electrochemical measurements, the CME, Ag/AgCl reference electrode and platinum auxiliary electrode were placed in the measurement cell via holes in the cover. Square-wave voltamograms were recorded using an EG&G Instruments Princeton Applied Research Model 394 Electrochemical Trace Analyzer. A frequency of 4 hertz was used along with a 50mV amplitude and 1 s repetition time for the instrumental settings. The electrodes were washed with DI water prior to any measurement to remove free-surface metal ions. Following rinsing with DI water, the CME was then placed into the measurement cell containing the supporting electrolyte where a potential of 0.5 V was applied and scanned to a final potential of - 0.9 V. The CME was scanned before preconcentration to obtain background measurements prior to preconcentration studies. Copper solutions were made from a Cu(II)SO4 salt with DI water and the solution pH was adjusted using dilute HCl as required. All solution concentrations were verified by flame atomic absorption spectroscopy. For preconcentration, the CME was immersed in the 20-mL copper solution (stirring) for a predetermined period of time with an open circuit on the CME. Following the preconcentration step, the CME was rinsed with DI water and then placed into the measurement cell. After the voltammetric measurement, the electrode was transferred to the cleaning cell for 10 minutes to remove the bound metal ions and then transferred again to the measurement cell containing fresh electrolyte solution where it was scanned again to verify the removal of the adsorbed copper. Each experiment was performed in triplicate with a new CME to provide a fresh surface for analysis, with the exception of the stability experiment where the same electrodes were used repeatedly. The averages of these data are reported herein.

RESULTS AND DISCUSSION

Previously performed experiments have shown that alfalfa biomass has a high affinity for copper ion binding (Gardea-Torresdey et al., 1996b;1999c). However, due to the lack of knowledge regarding the binding mechanism of the copper ions by the alfalfa biomass, we have chosen to perform voltammetric experiments to gain further insight into this mechanism. Voltammetry is a widely employed method by many researchers to study adsorption processes on surfaces. Carbon paste-modified electrodes have been used by several researchers to investigate the interactions of metal ions with different materials (Gardea-Torresdey et al., 1988; Wang et al., 1992; Kula et al., 1999; Lubert et al., 1999). In order to perform

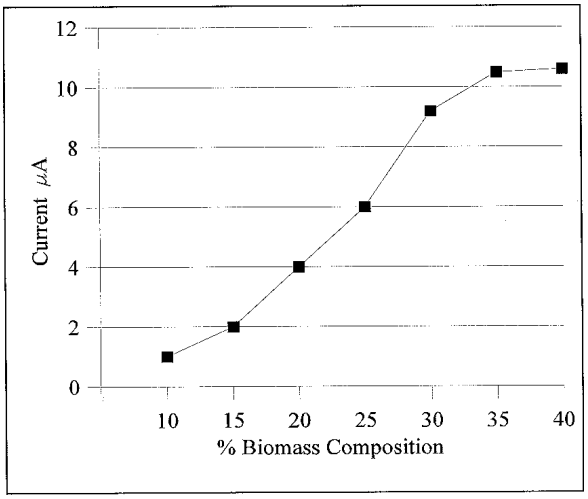

Figure 1. Effects of peak current obtained from various biomass/carbon paste compositions upon exposure to 0.1 mM Cu(II) at pH 5.0 with a five-min. preconcentration time.

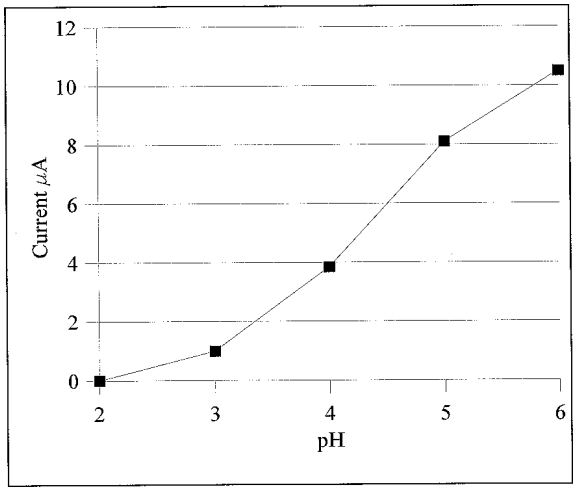

Figure 2. Effects of preconcentration solution pH on peak current obtained from the bioaccumulation of 0.1 mM Cu(II).

voltammetric experiments with the alfalfa biomass, an electrode containing the biomass was made. However, the amount of biomass needed to make the optimal paste composition needed to be determined. Figure 1 represents the voltammetric peak height current (μA) that was obtained upon preconcentration for five minutes with a 0.1mM Cu(II) solution at pH 5.0, and varied amounts of alfalfa biomass were used to make the electrode paste. The voltammetric peak height increases almost linearly as the percentage of biomass increases (10%-30%), as expected due to the increased binding capacity of the electrode. However, as the biomass composition is enriched above 30%, a leveling off of the voltammetric peak height was observed. This may be due to the reduction in conductivity of the paste with increased biomass composition. Therefore, further experiments were conducted with a carbon paste composed of 30% biomass.

The effects of preconcentration solution pH on the voltammetric peak height are shown in Figure 2, where a 0.1mM Cu(II) solution was used for the preconcentration solution at various pHs (2-6). As expected, the trend in voltammetric peak height increased respectively with pH and closely resembles that previously seen for the batch laboratory pH profiles for Cu(II) binding by alfalfa biomass (Gardea-Torresdey et al., 1996a; 1996b;1999c). At lower pH's, there was little voltammetric response, but from pH 3-4, there was a large increase in the voltammetric peak height, which began leveling off after pH 4, with a maximum achieved from pH 5.0 to 6.0. The decrease in voltammetric response is attributed to low adsorption of copper ions, thus indicating that the mechanism for adsorption is by a weak ion exchange via functional groups on the alfalfa biomass, which could be weakly acidic (such as carboxylic ligands) and protonated at pH 2.0.

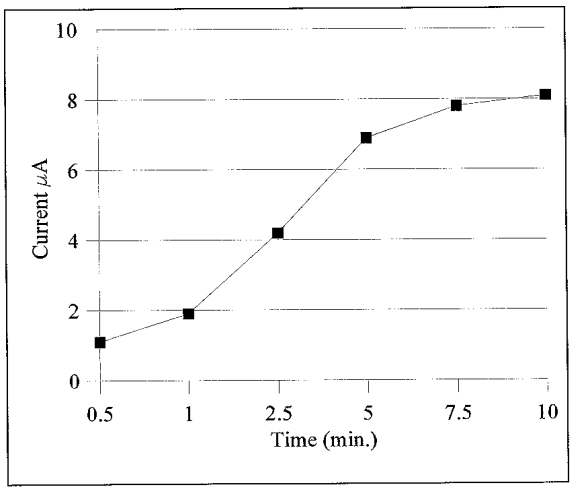

Figure 3. Dependence of preconcentration time on the peak current observed for the alfalfa-modified electrode exposed to 0.1 mM Cu(II) solutions at pH 5.0.

However, at higher pH values, the coordinating ligand may be deprotonated and available to bind positively charged ions such as Cu(II). There is an excellent correlation between the trend observed in Figure 2 and the batch laboratory pH profile work previously performed (Gardea-Torresdey et al., 1996a). Since carboxylic groups generally have pK values between 3 and 4, they are most likely responsible for the Cu(II) adsorption as previously found for Ni(II) and Cr(III) binding by the alfalfa biomass (Gardea-Torresdey et al., 1996b; Tiemann et al., 1999).

Further information regarding the optimal time of exposure was obtained by varying the preconcentration time of the CME with the 0.1mM Cu(II) solution at pH 5.0. Figure 3 shows the increasing voltammetric response acquired through extended accumulation times in the preconcentration solution. As the accumulation time increases, the response rises slowly within the first minute, followed by a steady increase up to five minutes, and ultimately begins to level off after seven minutes. More than 80% of the final response was generated within the first five minutes of exposure. These findings are in agreement with batch laboratory, time-dependent experiments which have indicated that the majority of the Cu(II) accumulation takes place within the first five minutes of contact with the alfalfa biomass (Gardea-Torresdey et al., 1996a). Because of the rapid accumulation of Cu(II) at the electrode surface within the first five minutes of preconcentration, the subsequent experiments were performed with a preconcentration time of five minutes.

Since the alfalfa biomass from the CME has shown to have a strong pH-dependent affinity for Cu(II) as displayed by the voltammetric response obtained in Figure 2, lowering the pH of the cleaning solution with 0.1M HCl should remove the Cu(II) bound from the electrode surface. Therefore, renewal of the electrode surface was accomplished by immersing the electrode in 0.1M HCl for a period of 10 minutes, and by the subsequent voltammetric run which showed no copper peak (data not shown). The effective cleaning and renewal of the electrode surface is shown in Figure 4, which displays peak current obtained from a series of 12 repetitions of accumulation and cleaning with the same electrode surface. As seen in Figure 4, a peak current of 9μA was obtained for the first two runs, which diminished to a steady mean peak current of 6.08μA. These results indicate that the electrode has a stable surface where the Cu(II) is binding and

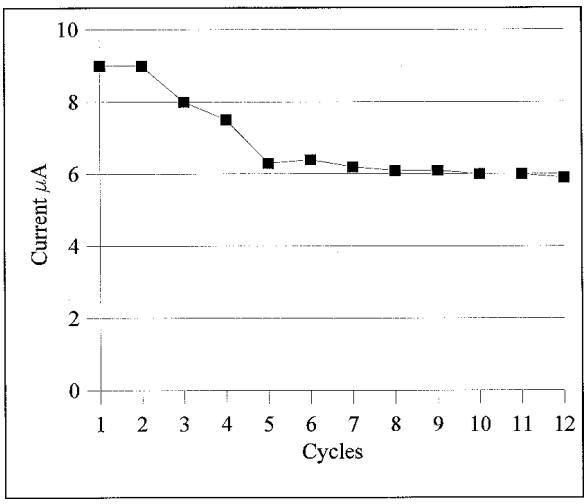

Figure 4. Effects of sorption/stripping cycles on the peak height using a single electrode. The electrode was exposed for five minutes to a preconcentration solution of 0.1 mM Cu(II) at pH 5.0 scanned, and then cleaned with 0.1 M HC1 for 10 min.

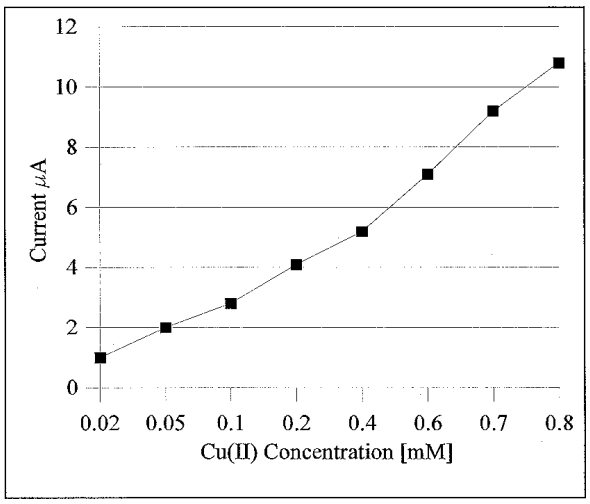

Figure 5. Effects of varying Cu(II) concentrations used for the preconcentration solution at pH 5.0 on the voltammetric response of the alfalfa-modified carbon paste electrode.

the mechanism for the majority of the binding is through a reversible process, most likely via an ion exchange-type process through carboxylic ligands.

The CME's voltammetric response to varying concentrations of Cu(II) preconcentration solutions is shown in Figure 5. As can be seen from Figure 5, the response is linear from 0.02mM Cu(II) to 0.8mM Cu(II) (1.27 ppm to 50.8 ppm) with a correlation coefficient of 0.99. The response began leveling off beyond the shown data points, indicating the saturation of the alfalfa biomass binding sites at the electrode surface. These results indicate that chemical binding sites on the alfalfa biomass have a high affinity for the copper ions in solution and the amount of accumulation is a function of surface area, which demonstrates that the accumulation of Cu(II) is only at the exposed electrode surface and Cu(II) does not leach into the inner electrode paste. Therefore, the binding of Cu(II) by the alfalfa biomass is most likely through functional groups located on the surface of the tissue cell walls, since the accumulation did not increase with preconcentration time (Figure 3) and solution concentration.

The experimental results obtained have shown that the use of electrochemical methods can provide useful information to help elucidate the binding mechanisms of biomaterials. Through voltammetric measurements via modified carbon paste electrodes, the effects of varying preconcentration times and solution pH have indicated that the accumulation of Cu(II) by the alfalfa biomass is occurring through surface functional groups via an ion-exchange type process and most likely involves carboxyl groups. In addition, the data have shown that the binding mechanism is most likely to be a reversible process. Therefore, the use of alfalfa

biomass to recover copper ions from contaminated waters by way of a reusable, cost-effective method would hold great promise.

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