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Impact of Surface Modification on Binding Affinity Distributions of *Datura innoxia* Biomass to Metal Ions

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Datura innoxia biomass has previously been targeted as a promising sorbent for heavy metal reclamation and remediation. Although the metal sequestering capabilities of biomaterials have been known, a general inability to accurately predict their binding characteristics has been a barrier to their implementation in remediation schemes. Of interest in our laboratory are the binding mechanisms as well as basic chemistry involved in the metal uptake of the material derived from fragments of cell walls from cultured cells from the plant *D. innoxia*. Previous studies have suggested the dependency of lead-ion affinity distributions of the biomaterial upon solution variables (i.e., ionic strength, pH) to be the result of two classes of binding sites: low-affinity sites involving sulfonates and carboxylates in an ion-exchange process and high-affinity sites resulting from the coordination of carboxylates to the metal. This hypothesis has been further investigated by the removal of the dominant functionality on the biomaterial (carboxylates) by carboxyl esterification by mildly acidic methanol. The resulting modified biomaterial was then studied in terms of affinity distributions toward proton and lead ions. The content of sulfonates on the biomaterial was found to be 43 μmol/g. A total amount of carboxylates involved in Pb^{2+} binding was found to be 560 μmol/g with 147 μmol/g involved in an ion-exchange mechanism and coordination sites including 212 μmol/g biomaterial.

Introduction

Global awareness of the underlying detriment of heavy metals in the environment has promoted research efforts toward alternative remediation technologies that are economical and environmentally sound. One of the promising technologies which has been investigated involves the use of plants and microorganisms to scavenge toxic metals (*1*−*4*). Although living biomasses have been explored and evaluated (*5*, *6*), nonliving biomasses (*7*, *8*) have also been studied for possessing several unique characteristics for contaminated water treatments, including the capability to be utilized under adverse conditions (*1*). One of such nonviable biomasses, which has been targeted in our research, is derived from anther cells cultured from the plant *Datura innoxia*. It was chosen because it has shown the ability to accumulate various heavy metals with adequate capacities and the feasibility to be cultured with high biomass production (*9*−*13*).

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In nonviable biomaterial systems, there are several parameters which can influence metal-binding capacities and selectivities. These parameters include environmental chemical conditions [e.g., pH, ionic strength, the presence of other ions (*14*, *15*)] and the properties of biomaterials, such as chemical heterogeneities and variable charges (*16*−*18*). Consequently, to maximize the efficiency of biomaterials and to improve their traits for heavy metals remediation, an understanding of their metal uptake mechanisms and the basic chemistry involved in the metal-binding processes has become essential.

Biomaterials normally contain certain quantities of proteins, lipids, and carbohydrates. These biomolecules and organic compounds can provide chemical functional groups that could act as metal-binding sites. Functionalities which have been proposed to be involved in metal sequestering have included carboxyl, sulfonate, sulfate, phenolic, phosphate, sulfhydryl, and amide moieties (*19*). Among them, carboxyl, sulfonate, and phenolic have been confirmed to be prevalent for many biomaterials (*7*, *11*, *16*). Also, mechanisms regarding metal sorption processes have been suggested to primarily encompass ion-exchange and adsorption processes (e.g., complexation, coordination, or chelation) (*20*). Due to the difficulties resulting from the inherent chemical heterogeneities of the biomaterial systems, several models have been developed in an attempt to describe overall metal-sorption behaviors under variant conditions. Generally, these models can be divided into two categories: discrete-site and continuous-site models (*21*−*23*). Considering the complexity of the biomaterials, continuous-site models are thought to provide a better description of metal sorption behaviors of such systems (*22*).

An elucidation of metal-ion sorption processes of nonviable anther cells cultured from the *D. innoxia* plant has been the focus of our research (*9*−*13*). These studies have included the applications of Eu(III)-luminescence and NMR for the characterization of the binding sites for metal ions. Results of these investigations have shown carboxylates to be responsible for the majority of metal-ion binding, especially at higher pH conditions (e.g., pH 4−5.5). Measurements from Eu(III)-luminescence spectra have also indicated the possible formation of different interactions between Eu^{3+} ions and carboxylates, including as mono-, bi-, and tridentates (*24*). Sulfonates (or sulfates) were reported to be involved in metal-ion binding with more pronounced contributions at lower pH (i.e., <3) (*24*).

The determination of binding capacities and affinities (i.e., affinity distributions) of these sites has also been undertaken. These parameters are important for the prediction of metal-ion binding under different chemical environments. In a previous paper (*25*), studies of apparent affinity distributions of the immobilized *D. innoxia* to Pb^{2+} ions at variant solution compositions were described. Two classes of binding sites between Pb ions and the biomaterial were identified. One class exhibited low affinities (mean affinity constant about 200 M^{-1}) and the other displayed higher affinities (mean affinity constant around 10^5 M^{-1}, depending on solution conditions). Preliminary experiments of the affinity distributions with varied pH suggested the low-affinity sites involved both carboxylate and sulfonate functional groups, while the high-affinity sites involved primarily carboxylates.

The further testing of these hypotheses through chemical modifications of the biomaterial substrate is the subject of the present study. The removal of the dominant chemical functionality would be predicted to significantly alter the

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measured affinity distributions. In this study, esterification of carboxylates on *D. innoxia* cell was performed. The formation of the resulting methyl esters and the impact of the modification process on the intactness of major structure of the biomaterial was investigated spectroscopically using FT-IR and ^{13}C NMR. The degree of esterification was measured by quantitation of methanol released from the base hydrolysis of the modified material. Comparisons between immobilized native and esterified cells were made with respect to metal binding abilities and site distributions for protons and metal (i.e., lead) ions.

Experimental Section

Materials and Chemicals. Fragments of anther cells cultured from the plant *D. innoxia* were selected as the biomass studied. The conditions under which these cells were cultivated have been described elsewhere (*26*). Prior to its use, the biomaterial was subjected to an acid wash (5% H_2SO_4 adjusted to pH 2 with 1.0 M NaOH) and subsequently rinsed with distilled, deionized water (D/D H_2O). The biomaterial was then freeze-dried and stored.

Carboxyl groups of the biomass (from a portion of the dry materials) were esterified using Fischer esterification reaction with methanol in the presence of a strong acid (*27, 28*). Specifically, a 13.0 g sample of the *D. innoxia* material was suspended with continuous agitation in a 750 mL anhydrous methanol (VWR), to which 6.5 mL of concentrated HCl (36%) was added to make the mixture in 0.1 M HCl (*12*). Samples with varied degrees of esterification were collected at 6, 24, 48, 54, and 74 h, respectively. All of the collected samples were washed to quench the reaction with excess D/D H_2O immediately after their removal from the esterification process and then freeze-dried and stored.

^{13}C label methanol (Sigma) was also used for the esterification of cell material. The resulting modified cells were later analyzed by ^{13}C NMR. In this case, only 200 mg of the biomaterial was subjected to the esterification for 48 h.

Measurement of methanol released during base hydrolysis of the esterified samples was conducted to test the extent of the esterification. A procedure similar to that reported elsewhere (*12*) was employed to perform the hydrolysis. Basically, 35 mg of each esterified sample including one from native cells was suspended and agitated in a 2.5 mL of 0.25 M NaOH for 5 min and then stored overnight at 4 °C. The samples were centrifuged, and 100 μL of each supernatant was transferred to a 1 mL volumetric flask and diluted with Nanopure H_2O. Methanol released during the hydrolysis was determined via gas chromatography (GC) (Hewlett-Packard 5880A). The GC with a RSL-500 polar column was operated using column temperature of 40 °C, an injector temperature of 250 °C, and a flame ionization detector temperature of 270 °C. Before injection, each sample was spiked with 0.06 mM 1-butanol to serve as an internal standard.

Portions of the dried native cells and the esterified cells (74 h of esterification) were each immobilized within a polysilicate matrix according to the procedure described elsewhere (*26*). As discussed previously (*13*), only the 40−60 mesh fraction of the resulting immobilized biomass was used.

A stock solution (2.5 mM) of lead was prepared from its nitrate salt with the sodium form of 2-(*N*-morpholino)-ethanesulfonic acid (MES) buffer (Sigma) of desired concentration in D/D H_2O (or Nanopure H_2O from Barnstead Thermolyne D4751). The MES was chosen because of its inability to chelate metal ions (*26*). All influent solutions used for affinity chromatography were prepared from the stock solution by serial dilution using this buffer solution at the same concentration and were subsequently adjusted to the desired pH (model 710A, Orion).

Analytical and Mathematical Methodology. For non-immobilized native and esterified cells, metal-binding abilities were studied in a batch mode. For these studies, a 35 mg sample of each material was mixed with 10 mL of the metal-ion solution. This contact solution was 1.0 mM Pb^{2+} and 0.01 M Na-MES at a pH of 5.5. After 2 h of agitation for equilibration, the sample was centrifuged. The metal content in the supernatant was analyzed via an inductively coupled plasma (ICP) emission spectrometer (Jarrell-Ash Div. of Fisher Scientific Company, AtomComp Series 800). The amount of metal bound to each cell sample was calculated by subtracting the metal-ion content of the supernatant from the initial metal content of the contact solution.

Modified frontal affinity chromatography (*13*) was employed to obtain Pb-binding isotherms on both immobilized native and esterified cells. Briefly, in this modified mode, an influent Pb solution was applied continuously by a peristaltic pump (Gilson Minipuls 2) to a column. The column was packed with the immobilized biomaterial (usually 35 mg) and was preequilibrated with the buffer solution at the same ionic strength and pH as the influent. After passing a sufficient volume of the influent to the column (*13*), the metal content in the collected effluent was determined using either a flame atomic absorption spectrometry (FAAS, Perkin-Elmer 3030B) or ICP emission spectroscopy. The FAAS and ICP were each operated according to the manufacturers recommended procedures. From the difference in the metal content between the influent and effluent, the amount of metal bound in equilibrium with the influent solution was determined. A series of the influent solutions with a wide range of metal-ion concentrations were repeated in the same manner. A binding isotherm with a typical relative standard deviation of 1∼5% was then generated.

Affinity distributions were determined from the lead-ion-binding isotherms using regularized least-squares regression analysis by applying the computer program QUASI. This program has been described in detail elsewhere (*29*). Typical program parameters used in this study included a "spectral" window of log *k* from 1.5 to 6.5, a grid spacing of 0.1, and a regularization parameter of 1. The nonnegativity constraint and the smoothness regularizing function were also employed. No constant or linear isotherm was included when using this program.

Acid−Base Titration. Titration of both the immobilized native and esterified cells was conducted through the same procedure as described elsewhere (*30*). Briefly, 35 mg of each sample (40/60 mesh) was suspended in 25 mL 0.01 M $NaNO_3$ and degassed with ultrapure argon. Standard acid (HNO_3) and base (KOH) solutions were prepared from Dilut-It (J. T. Baker) analytical concentrates diluted with D/D and degassed H_2O. All potentiometric equilibrium measurements were taken under an atmosphere of ultrapure argon in a water (from 25.00 ± 0.02 water bath) jacketed titration vessel. Titrations were conducted using a Radiometer ABU-93 combination triburet and pH/pX meter outfitted with a 1 mL buret (acid) and a 5 mL buret (base). An Orion 91-02 glass combination pH electrode was employed. The autoburet/pH meter was controlled by a computer using software (ALPENGLOW) developed in Los Alamos National Laboratory(*30*).

FT-IR and NMR Spectra. Infrared (IR) spectra were obtained and averaged over 8 scans at a resolution of 4 cm^{-1} with a Fourier transform infrared spectrometer (Perkin-Elmer FTIR 1720X). Sample disks were made from 5 mg of *D. innoxia* encapsulated in 150 mg of KBr. As described previously (*12*), this ratio produced a better resolved IR spectra than the 1:100 typically recommended.

Solid-state ^{13}C NMR spectra of both native and ^{13}C label esterified cells were obtained according to the manufacture recommended procedure, including the use of a 5 mm VT

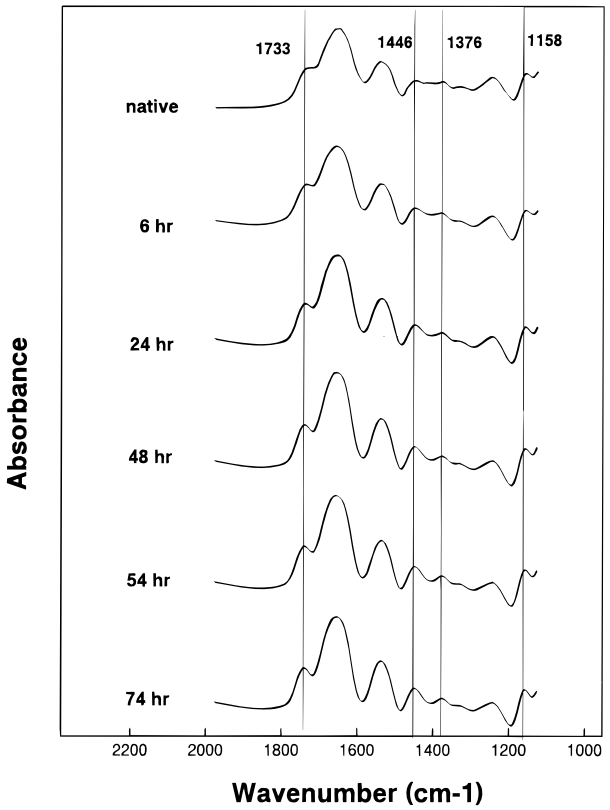

FIGURE 1. FT-IR spectra for *D. innoxia* cell fragments with varied degrees of esterification, including 0, 6, 24, 48, 54, and 74 h, respectively. Vertical lines indicate specified absorption bands.

CP/MAS probe on a Varian Unity 400 spectrometer with a 9.4 T narrow-bore superconducting magnet.

Results and Discussion

The esterification reaction performed to modify the fragments of *D. innoxia* cells can be simply expressed as:

$$R{-}COOH + CH_3OH \overset{H^+}{\rightleftharpoons} R{-}COOCH_3 + H_2O$$

where R represents all components in the biomass, except for carboxyl groups which are native to the biomaterial. To verify such modification toward the cell fragments, both of FT-IR and ^{13}C NMR spectroscopies were employed to monitor formations of absorption bands corresponding to ester carbonyl and/or methoxy groups which are the indications of esterification reaction.

The FT-IR spectra in 2000−1100 cm^{-1} regions for native *Datura* cell fragments and cell fragments with varied periods of esterification (6, 24, 48, 54, and 74 h, respectively) are shown in Figure 1. Band assignments are based according to refs *12* and *31*−*33*. The unresolved shoulder near 1738 cm^{-1} was assigned to the stretching vibration of ester carbonyl groups for all esterified cells except for native cells. The shoulder band appearing at lower frequency (~1733 cm^{-1}) for native cells can be due to the combining contribution from both of carbonyl stretching vibration of carboxylic acid and ester native to the biomaterial. The bands near 1446 and 1376 cm^{-1} can be ascribed to the symmetric C−H bending vibration of end-methyl groups, mainly including $-O-CH_3$ and $-C-CH_3$, respectively. Similarly, the absorption band near 1400 cm^{-1} in the valley between bands at 1446 and 1376 cm^{-1} can be attributable to symmetric carboxylate vibration. The dominating bands near 1656 and 1540 cm^{-1} are assigned to amide I and amide II, the characteristic IR absorption of protein which could be one of the significant components of cell walls. A substantial portion of the absorbance at 1158 cm^{-1} is attributable to the stretching vibrations of C−O on the ring structure of carbohydrates, the main components of the cell walls. There does not exist a unanimous agreement of assignments regarding the absorbance peak around 1240 cm^{-1} which could be due to the vibration contribution from C−O−S stretching of sulfate (*7*), the C−O stretching of carboxyls (*33*), or phosphate stretching (*30*).

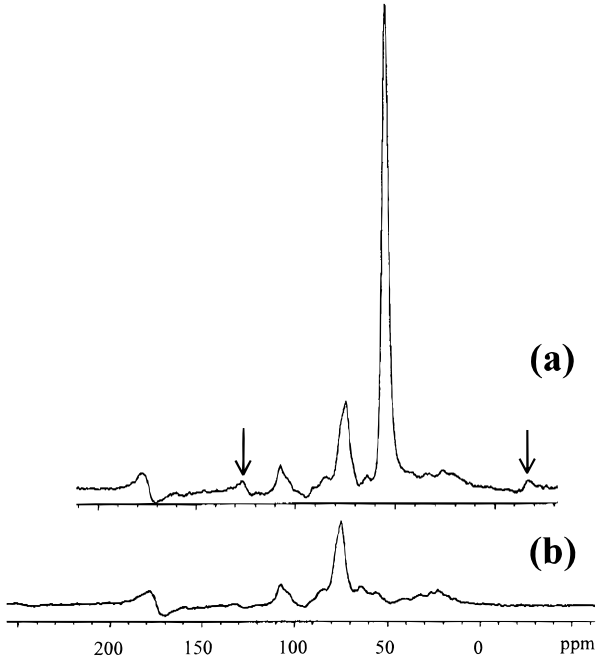

FIGURE 2. ^{13}C NMR spectra for (a) cell fragments esterified using ^{13}C-enriched methanol and (b) native cell fragments. Arrows designate spinning side-band artifacts.

From these IR spectra, it is observed that both of the absorbances around 1738 cm^{-1} (ester carbonyl) and 1446 cm^{-1} (methoxyl) increased with increasing extent of esterification (i.e., reaction time), while that at the valley near 1400 cm^{-1} (carboxylate) decreased with exposure to acidic methanol. These results verify that the carboxyl esterification of the biomaterial has been undertaken by using the Fischer process. Also, it is observed that the band intensities at 1738 and 1446 cm^{-1} barely increased after 48 h esterification, suggesting that the esterification reaction had either reached the equilibrium or was near completion. Because of the use of a large excess of methanol, the reaction completion is a more plausible explanation. In addition, absorbances of amide I, amide II, and that at 1158 cm^{-1} (C−O stretching vibration, mainly from carbohydrates) were nearly unaffected under these esterification conditions. This indicates that, after esterification reaction, the major components and the structure of the biomaterial remained intact. This observation can be further confirmed by use of ^{13}C NMR spectra of the modified cell fragments.

A comparison of ^{13}C NMR spectra for native cell fragments and cell fragments esterified using ^{13}C-enriched methanol is presented in Figure 2. The chemical shift (ppm) is given approximately relative to tetramethylsilane (TMS). Resonance peaks are assigned according to ref *51*. The subtle difference of the peak in the range of about 50−58 ppm is attributable to the absorption of the carbon from $-O-^{13}CH_3$ groups bonded to carbonyl group of esters, though, the broad feature of this peak prohibits the distinguishing of the possible contribution of end methyl groups from ether to this resonance. Also, there is a slight chemical shift in the carbonyl region (170−185 ppm) of the spectrum for the modified material due to the conversion of carboxyls to esters (*34*).

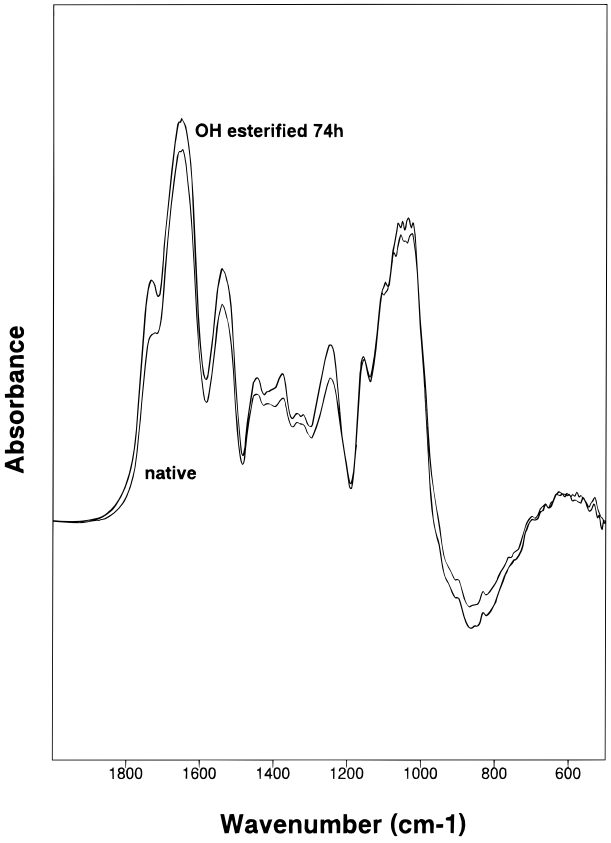

FIGURE 3. The correlation between the amounts of Pb bound by native and esterified biomaterials (■) and the corresponding amounts of methanol released (●).

Both of these results further confirm the esterification. However, except for this sharp difference, there are no other obviously detectable variations between these two spectra (excluding two symmetric sidebands caused by magic angle spinning inherent to the solid-state NMR technique). These data further support the conclusion obtained from IR study that the primary structure of the biomaterial exhibited no significant change upon esterification. Therefore, any pronounced change in metal-binding ability of the modified biomaterial should be attributable mainly to the modification of the functional groups rather than to the structure change of the biomaterial.

The effect of esterification on the metal-binding ability of the biomaterial was studied by measuring the amount of Pb bound to a series of modified samples. The quantity of methanol released during the base hydrolysis of the modified biomaterial is equivalent to the content of carboxyl groups which have been esterified. These results are presented in Figure 3 by correlating the Pb-binding abilities of the biomaterials with the corresponding amounts of methanol released when the biomaterials are subjected to base hydrolysis. Results shown are for native cell fragments not exposed to esterification and for cell fragments esterified for certain period of times, including 6, 24, 48, 54, and 74 h. For cell fragments esterified for less than 24 h, it is observed that there was a dramatic decrease of Pb uptake after esterification along with a good correlation between the amounts of metal bound and the amounts of methanol released: the decreased amounts of Pb bound were nearly proportional to the increased amounts of methanol released. These results verify that carboxyl groups are important and the dominating moieties responsible for metal binding by the biomass. Meanwhile, the observed decrease in Pb uptake became less dramatic after 24 h and nearly unchanged after 48 h. These

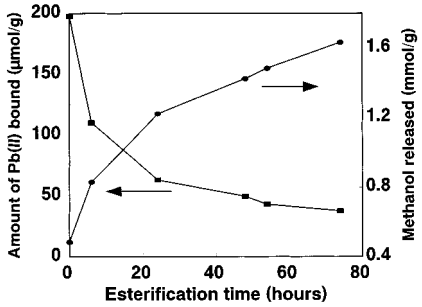

FIGURE 4. The comparison of FT-IR spectra for native cell fragments (solid line) and the hydroxyl esterified cell fragments (dashed line).

data might suggest that carboxyl esterification reaction approached completion after 48 h of esterification, which agrees with the IR results as discussed above. Although carboxyl groups account dominantly for the metal binding, the nonzero Pb uptake after 48 h of esterification suggested that other functionalities are also involved in metal binding, which would be consistent with the proposed involvement of sulfonates (*9*, *10*). Unlike the change trend of the metal uptake by the modified biomasses, the measured amount of methanol released continued to increase even after 74 h of esterification. This result suggests that, besides carboxyls, there are functional groups on the *D. innoxia* biomass which could react, though at lower reaction rate, with methanol under the employed esterification conditions and that the resulting materials could reversibly release methanol upon base hydrolysis. Such functional groups also have minimal contributions toward metal binding to the biomaterial. It is therefore speculated that methyl etherification of hydroxyls on the biomass could have also occurred, though it should not be as favored as esterification under the conditions used. Although thiol groups would be plausible functionalities for metal binding, spectroscopic investigations of this material have not supported the involvement of this moiety (*9*−*12*).

To test this hypothesis that hydroxyls may play a minimal role in metal-binding capability of the biomaterial, a hydroxyl esterification was carried out by employing acetic acid in the presence of concentrated hydrochloric acid (*35*). Similarly, the formation of the absorbance band at 1738 cm^{-1} in FT-IR spectra (Figure 4) confirmed this reaction. The metal-binding ability of the cell fragments after such modification for 74 h was compared with that of native cell fragments free of modification. The results obtained by batch study indicated that the amounts of Pb uptake were similar for both biomasses (208.1 μmol/g for native cells and 208.7 μmol/g for modified cells). Thus, although hydroxyls may react with the methanol, they are not significantly involved in the binding of Pb^{2+} to the biomaterial.

From the above study of the integrated effect of the esterification on metal-binding ability of the biomaterial, it is now known that the greater extent of esterification the larger reduction of the binding capacity. As found before (*25*), the affinities of the immobilized *Datura* biomass to lead ions exhibited a distribution with two distinguishable classes of affinity sites. The question now arises as to how the esterification of the biomaterial affects the distribution of these two classes of binding sites. It is expected that the study of such effect on the individual class of binding sites will be beneficial for the elucidation of the involvement of carboxyls in each class of binding sites. Because the relationship between the metal complex formation constant (or affinity constant) and the pK_a of carboxylic acid could reveal the underlying bonding mechanisms for metal ions and carboxyls complexing system (*36*), the effect of carboxyl esterification on proton affinity distributions of the immobilized *Datura* biomaterial was thus investigated for the extraction of the pK_a values of carboxyls on such materials.

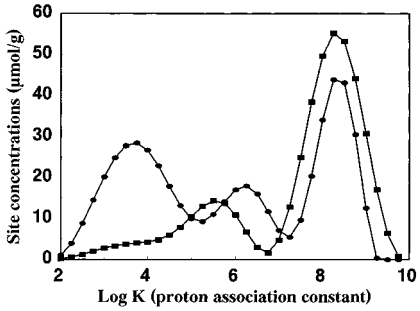

FIGURE 5. Proton-affinity distributions for both immobilized unesterified (●) and esterified (■) cell fragments in a 0.01 M $NaNO_3$.

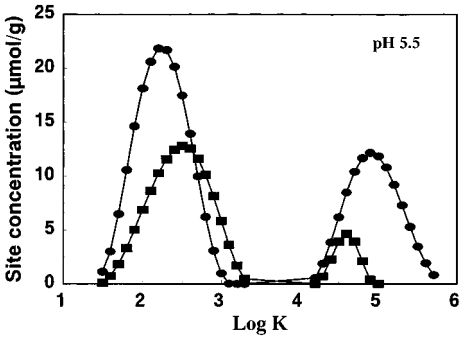

FIGURE 6. Comparison of lead-binding affinity distributions at pH 5.5 to the immobilized native (●) and esterified (■) biomaterials.

Applying the same methodology as described previously (*25*), proton-affinity distributions on the immobilized biomaterials were obtained from the corresponding proton binding isotherms using the QUASI program. Proton-binding isotherms were deduced from the acid titration curves of the biomaterials with titration ranging from pH 10 to 2.5. Figure 5 presents the results of proton-affinity distributions for both unesterified and esterified immobilized cell fragments in 0.01 M $NaNO_3$. As can be seen from Figure 5, after modification, there is a great reduction in the first distribution peak (first class of proton-binding sites). IR and NMR results, as discussed above, have indicated that the biomaterial experienced no apparent structure changes upon modification, excepting carboxyl esterification and speculated hydroxyl etherification (methylation). Comparing the proton association constants (log K in Figure 5) of the first class of proton-affinity sites with literature pK_a values of both carboxyl and hydroxyl groups (*37*), it is reasonable to assign this class of proton affinity sites as carboxylates. Moreover, the wide span of the log K values (from log $K = 1.9$ to 4.8) of this class of binding sites reflects the variations of proton-affinity strength of these sites, and therefore, the existence of carboxylates in different molecular environments. Among them, the strongly acidic carboxyl groups (pK_a of 3.0 or less, a small portion of the first class of binding sites) may encompass carboxyl groups associated with amino groups. These have been considered because the pK_{a1} values of amino acids range from 1.8 ~2.6 (*38*), and they are present in the biomaterial. Also, a survey of pK_a values for organic acids (*39, 40*) indicates that a portion of strongly acidic carboxyl groups may involve polycarboxylic acids which are on aromatic rings or in aliphatic structures with electron-withdrawing groups (carboxylic acid, ketone, ester, ether, hydroxyl, and π-electrons in double and triple bonds) in proximity to the strongly acidic carboxyl group.

The weakly acidic carboxyl groups (pK_a of 3−5), which consist of a large portion of the first class of proton-affinity sites, may arise primarily from side-chain carboxy groups of amino acids in proteins or from monocarboxylic acids for their pK_a values falling in this range (*37, 38*).

It is worth noting that there is a small reduction after esterification in the second class of proton-affinity sites with log K extending from about 5 to 7. This could result from the removal of second carboxyl groups of polycarboxylic acids because a portion of the pK_{a2} values of these functionalities fall in this range (*39*). This suggests that the involvement of proton binding to the second carboxyl groups of polycarboxylic acids would account for a portion of second class of proton-affinity sites and the existence of polycarboxylic acids in the biomaterial. Because there is only a small reduction, this class of proton-affinity sites should not account for a significant change in the metal-binding-affinity distribution.

The comparison of lead-binding-affinity distributions at pH 5.5 to both immobilized native and esterified cell fragments is shown in Figure 6. Clearly, both classes of binding sites have been affected by esterification modification. Although the low-affinity sites (log K ranging from 1.5 to 3) were influenced to lesser extent, nearly all of high-affinity sites (log K from 4 to 6) were removed after esterification. These results further verify carboxyl groups to have been involved in both classes of binding sites. Moreover, these results appear to indicate that high-affinity sites only include carboxyl groups. Conversely, for low-affinity sites, besides carboxyls other functionality should have also been involved to account for the remaining sites after the removal of carboxyls. Combining with earlier results from Eu(III)-luminescence and NMR studies, this functionality is attributable to sulfonates (*9−11*). Confirmation of this conclusion can be performed through the modification of this functional group and which is another subject of future studies.

In studying the correlation between the formation constant of metal−ligand complex and the pK_a value of the corresponding monocarboxylic acid ligand (*36*), it was found that there was consistently a tendency for the former value to be smaller than the latter if the formed complex was only stabilized by simple electrostatic interaction. In contrast, the opposite holds when chelation forces are involved in the formation of the complex (*36*). Comparing results of Figure 6 with those of Figure 5, it was observed that, for the first class of Pb^{2+}−biomaterial complexes, the formation constants (affinity constants) were on a average smaller than the pK_a values of first class of proton-affinity sites (carboxylates). Therefore, based on these observations, it is reasonable to conclude that the low−affinity sites are accounted for the formation of unidentate complex between Pb^{2+} and the biomaterial, and this complex is stabilized only by simple electrostatic interactions. This verifies what was found previously (*25*): low-affinity sites involve primarily ion-exchange processes though electrostatic interactions.

As far as the high-metal-affinity sites was concerned, the majority of the ligand sites should be originated from the first proton-affinity sites. Because the log K values of the metal complexes were larger than the corresponding pK_a values, it is then concluded that chelation forces must have been involved in the formation of the metal complexes to consist of the high-metal-affinity sites. This again supports the previous conclusion (*25*) that adsorption process through chelation is attributed to the high-affinity sites of metal binding.

Figure 7 shows the comparison of lead-binding affinity distributions at pH 2.0 to both immobilized native and esterified cell fragments. Because the results in Figure 5 indicate that strongly acidic carboxyl groups are nearly removed after esterification, the contribution to the remaining site concentrations of the first class of binding sites result from sulfonate groups. Therefore, the content of sulfonates on the immobilized *D. innoxia* biomaterial is estimated to be 43 μmol /g.

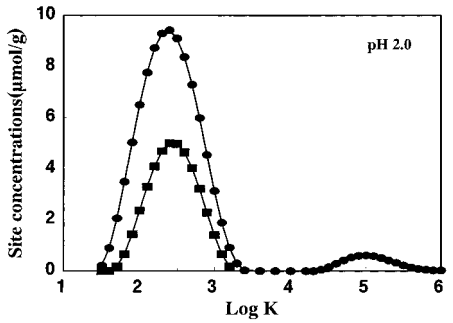

FIGURE 7. Comparison of lead-binding affinity distributions at pH 2.0 to the immobilized native (●) and esterified (■) biomaterials.

By the same manner of integration, the total-site concentration of carboxyl groups (in the form of either carboxylate groups or carboxylic acid, depending on solution pH) on the immobilized native cell fragments is estimated to be 560 μmol/g, while the Pb-binding capacities of both classes of low- and high-affinity sites at pH 5.5 are 190 and 106 μmol/g, respectively. Subtracting the contribution of sulfonates (43 μmol/g) to the Pb-binding capacity of low-affinity sites, the amount of carboxylates involved for low-affinity sites is around 147 μmol/g. Assuming that two carboxylates are chelated with one Pb ion (bidentate complex) to account for one high-affinity site, then, the amount of carboxylates involved in these sites is 212 μmol/g (twice the amount of Pb-binding capacity of the class of high-affinity sites). Summation of carboxylates involved in both class of Pb-binding sites gives the number 359 μmol/g. This is smaller than the total concentration of carboxyl groups on the biomaterial, which suggests that a portion of carboxyl groups was not involved in Pb binding. This is reasonable since certain part of carboxylates is still associated with protons at this pH condition (pH 5.5).

Acknowledgments

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