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Impact of pH on Binding Metal Ions by *Datura innoxia* Biomass

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ABSTRACT

Metal ion binding to biomaterials derived from cultured antheral cells of *Datura innoxia* has been investigated as a function of solution pH. Specifically, the binding of Ba^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Sr^{2+} was studied at pH 1–6, inclusive, in 0.1 M MES solutions. For 10 $mg \cdot L^{-1}$ metal ion solutions, maximum binding was observed for solutions at pH 5. The dependence of metal ion binding on solution pH was studied for the biomass both free in solution and immobilized in a polysilicate matrix. A significant change in the pH-dependent binding of each of these metals was observed when the cell material was immobilized. These variations in the impact of metal solution pH could not be explained by the Lewis acidity of the metal ions. The involvement of conformational changes of the macromolecular components of the material for the formation of metal-selective coordination sites is postulated.

INTRODUCTION

The use of biologically derived materials as sources of metal binding for wastewater treatment has been amply studied (1–10). The majority of

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these investigations have, however, focused on the use of algal biomasses. Both living and nonviable algal systems have been investigated (1–10). Unfortunately, the overall efficiency of metal binding by living cells has been reported to be unpredictable and unstable (11). Because of the limitations present with living organisms, nonviable (i.e., dead) cell systems have been demonstrated to be a better substrate for metal binding (12).

Living cells bind metals in various ways. They can ingest metals by means of active transport. Metals can enter the living cells during pino- or phagocytosis, and they can be adsorbed to the surface of the cell wall. The drawback to this is that living cells will not actively transport metals which are toxic to their systems, nor will they actively ingest too much of a nutrient metal. Thus, if the algae are passively ingesting either excess nutrient metals or toxic metals, the system should become at least partially dysfunctional.

Studies of the optimum conditions binding toxic metals by dead antheral cells from *Datura innoxia* have been undertaken (13–17). *Datura innoxia*, more commonly known as *Datura meteloides* or Sacred Datura, is a plant which is indigenous to Mexico and the southwestern United States. *D. innoxia* was selected because it belongs to the well-studied family *Solanaceae* (e.g., potatoes, tomatoes, and tobacco) and is tolerant of many heavy metals (18). This tolerance is demonstrated by its natural growth in alkaline and heavy-metal-rich soils.

The antheral cells from the plant were selected because of the increased probability of isolating a pure cell line. Because of the innate complexity of higher ordered (i.e., multicelled) plants, it was desirable to investigate the binding of metals to cell-wall functional groups without the complication of multiple types of cells. A mixture of two types of antheral cells was isolated for these studies. This mixture of fragments of these cells comprised the biomass used in this work.

The objective of this study was to phenomenologically ascertain the impact of solution pH on the relative efficiency of metal ion binding to this novel biomass. Parallel studies have been conducted in this laboratory using the cells in their free form and immobilized in a polysilicate matrix. Immobilization of the cell fragments was required to enable the subsequent application of this material in a packed column. Previous applications of algal biomasses have demonstrated the utility of such an immobilization procedure to flowing systems for water treatment (6, 19). It is, however, beyond the scope of the present discussion to adequately address the impact of the immobilization procedure on the efficiency of metal binding. This topic is addressed elsewhere (20) and is the topic of ongoing investigations.

EXPERIMENTAL

Two grams of the finely divided cultured cell fragments were mixed with 35 mL of 0.01 M HNO_3 (pH 2). These mixtures were agitated for 1 hour and centrifuged at 15,000 rpm for 15 minutes. The acid wash was repeated once and followed by a rinse with distilled deionized water. This washing procedure was undertaken to remove exchangeable endogenous metal ions and soluble biomolecules. Finally, the washed *D. innoxia* biomass was freeze-dried. The clean cell material was ground to a fine (100 to 200 mesh) powder and stored for later use in a plastic container. The conditions under which the cells were cultured have been described elsewhere (13).

A portion of the cell material was immobilized in a polysilicate matrix according to the procedure generally described elsewhere (19, 20). Briefly, a 6% sodium silicate (as the pentahydrate salt) solution (Fisher) was added to 75 mL of 5% H_2SO_4 (Mallinckrodt) to achieve a solution pH of 2. Five grams of the sieved and washed cells were then added to the mixture. The slurry was subsequently agitated for 1 hour. The pH of the solution was slowly increased to 7.0 by the addition of the silicate solution. A polymerization of the silicate was observed. The polymer mixture was stirred for an additional 30 minutes and stored overnight in a refrigerator. After the cell-containing polymer had settled, the supernatant solution was removed. The material was washed several times with distilled deionized water until testing of the supernatant with a stock solution of barium nitrate failed to produce a visible precipitate. The final cell-polymer gel was then poured into several preweighed dishes and dried overnight at 87°C. The percentage of cell material within the polymer used in this study was determined by mass balance to be about 80% by weight.

Stock solutions of 1000 ppm barium, cadmium, copper, nickel, and strontium were prepared from their nitrate salts. A 0.1 M 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer (Sigma) solution was prepared and used as the common matrix for all dilutions. This buffer was selected because of its demonstrated inability to complex with metal ions (21, 22). The pH of the buffer solutions was adjusted from 1 to 6 by using concentrated hydrochloric acid (Baker) or sodium hydroxide (Mallinckrodt). All solutions were prepared daily by serial dilution with distilled deionized water.

Metal analyses were conducted using flame atomic absorption (AA) and inductively coupled plasma (ICP) atomic emission spectrometry. The AA spectrometer was a model 457 (Thermo Jarell Ash) with an air-acetylene flame. The ICP spectrometer was a hybrid instrument designed in our

laboratory. This emission spectrometer has been described elsewhere (13, 23). Briefly, it utilizes a Plasma Therm (RF Products) rf power supply and a cross-dispersion Echelle monochromator with a symmetrical-arm over-and-under off-axis spherical mirror pair used for image transfer between the plasma and the entrance slits of the monochromator. Emission was detected by an end-on photomultiplier tube (model R292, Hamamatsu) operated at −900 V. The plasma was operated at 1000 W with outer, intermediate, and sample gas flow rates of 18, 1.0, and 0.8 $L \cdot min^{-1}$, respectively.

Metal solutions (10 $mg \cdot L^{-1}$) were prepared from stock solutions and pH 1 through 6 MES buffers. The actual pH of each metal/buffer solution was measured and recorded. The primary purpose of the MES was not for its buffering capacity but to control the ionic strength of the solution without introducing competing complexation equilibria. Three milliliters of metal solution were added to triplicate 30 mg samples of the free or immobilized cell materials (i.e., a total of 36 samples). The samples were agitated for 20 minutes and then centrifuged for 10 minutes using a benchtop centrifuge. The pH of each supernatant was measured and recorded for later comparison with the original solution pH. A 1.0-mL aliquot of a 10,000-$mg \cdot L^{-1}$ sodium (as sodium chloride) together with distilled deionized water was added to each solution to matrix match the solutions at 1000 $mg \cdot L^{-1}$ sodium. The metal concentration in each solution before and after exposure to biomass material was determined by either AA (Cd, Cu, Ni) or ICP atomic emission (Sr, Ba) spectroscopy.

RESULTS AND DISCUSSION

The majority of the studies were conducted with metal solutions ranging in pH from 1 to 6. Both the free (Fig. 1) and immobilized (Fig. 2) cell material showed a somewhat sigmoidal relationship with respect to pH for most of the metals studied. Common to both types of cells was the observation that maximum binding occurred at pH 5. For 10 $mg \cdot L^{-1}$ solutions of nickel, copper, cadmium, and strontium, maximum binding occurred at pH 5 and minimum binding occurred at pH 1 or 2 (more commonly at pH 1). Barium binding to free cells produced similar sigmoidal curves and the common maxima and minima (Fig. 1). However, the data for Ba binding to immobilized *D. innoxia* differed in that it gave not a sigmoidal curve but a more parabolic-shaped curve, with a maximum at pH 5 (Fig. 2). The increase in binding to the cell material at pH 2 could be explained by the observed binding of Ba^{2+} to the silicate polymer. However, the availability of additional binding sites on the polymer would be expected to similarly affect the binding of barium ions at pH 1. The

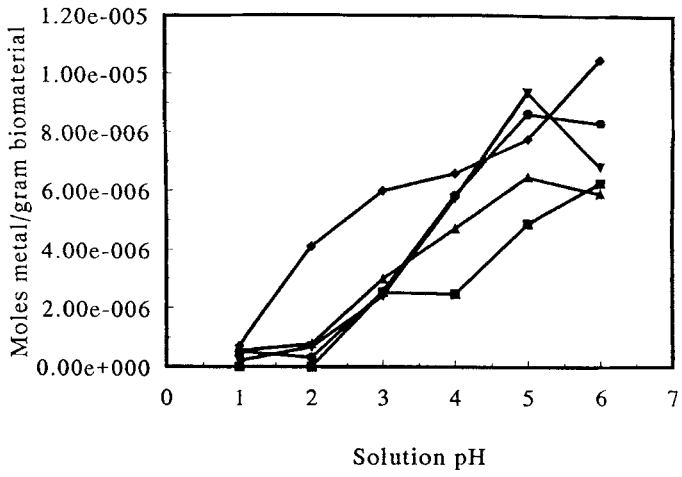

FIG. 1 Amount of metal bound to the free cell material per gram of cell material as a function of solution pH. All initial metal concentrations were 10 mg·L^{-1} (▲ Ba; ● Cd; ◆ Cu; ■ Ni; ▼ Sr).

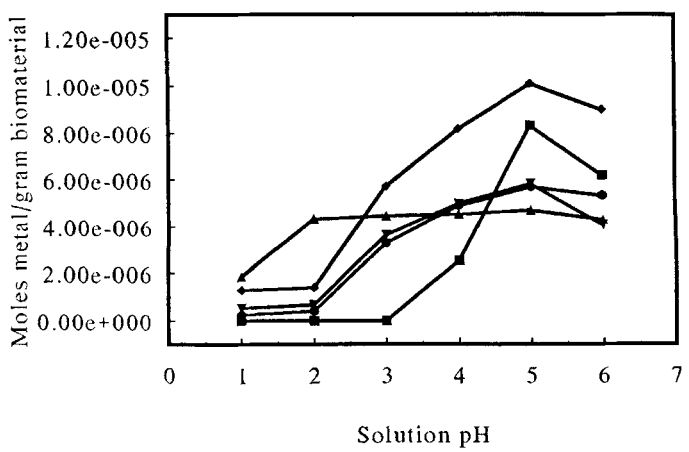

FIG. 2 Amount of metal bound to the immobilized cell material per gram of cell material as a function of solution pH. All initial metal concentration were 10 mg·L^{-1} (▲ Ba; ● Cd; ◆ Cu; ■ Ni; ▼ Sr).

enhanced binding to the immobilized biomaterial substrate at pH 1 does not fully support this argument. Interestingly, the removal of Ba^{2+} from the contact solutions at pH 5 and 6 decreased. Although strontium would be predicted to exhibit similar chemical behavior, a slight increase in binding to the immobilized cell material was observed for a solution of initial pH of 3 while a decrease in the amount of Sr^{2+} was measured for each of the other pH conditions studied.

Generally, the normalized binding of each of the metal ions investigated decreased at the higher solution pH conditions (i.e., pH 5 and 6). The binding of these species tended to remain low with low solution pH (1 and 2) with the exceptions of barium binding to the immobilized cell material and copper binding to the free cell material. The ability of these materials to bind metal ions at intermediate solution pH conditions exhibited no clear trends in terms of either chemical family (e.g., Ba^{2+} and Sr^{2+}) or Lewis acidity.

An interesting observation was made during the preparation of some samples of the free cells in the pH 1 and pH 6 buffer solutions. Upon comparison of the two samples it was noticed that the color of the cells was different. At pH 1 the cells were a light yellow color, but at pH 6 the color of the cells had grayed significantly. As subsequent studies were conducted, the color change was readily apparent. The graying was first visually noticeable as the solution pH was increased to 4 or 5. The color change, though not so dramatic, was also observed with the immobilized cells. It was more difficult to visually observe because the wet immobilized cells typically displayed a darker brown color than the free cells. Consequently, the color of the immobilized cell materials does not "gray" but merely darkens. This color change was reversible by alteration of the solution pH for both the free and immobilized cell materials.

Because there is a pH-dependent color change in the cells which appears to qualitatively correlate with the general pH dependency of metal binding, it was postulated that a conformational change of the molecular components of the biomass may be occurring. Although the biomass material has yet to be completely characterized, proteins, carbohydrates, and various other artifact cell components are suspected to be present (4). A series of studies designed to further explore this phenomenon has been undertaken in this laboratory. Variations in UO_2^{2+} luminescence (16) and ^{113}Cd-NMR (17) spectra with solution pH tend to support a mechanism involving conformational changes of macromolecular components of the biomaterial as the solution pH is altered.

Binding of the metals to a polysilicate blank (i.e., no cell material) was generally observed to be both pH-independent and relatively insignificant (<10% of maximum binding with the biomass material) except for the

binding of barium ions as previously discussed. The observed variations in the pH dependence of metal binding would suggest the possible alteration of the tertiary structure of macromolecules within the *D. innoxia* cell walls as a result of the immobilization process. This type of variation in the conformation of the molecular components of the biomaterial would be predicted to induce variations in the number and types of binding sites accessible to metal ions in cell material, thus enhancing the binding capacity for some metals at specific solution conditions while decreasing the ability of the material to bind other metal ions.

CONCLUSIONS

The binding of metal ions to a biomass material derived from cultured antheral cells from *Datura innoxia* has been observed to be pH-dependent. The amount of metal bound to the cell-material typically displayed a sigmoidal relationship as a function of increasing solution pH. Maximum metal-ion binding was generally measured at pH 5 to 6 for those systems displaying that behavior. An exception to this sigmoidal pH-dependence was the binding of Ba^{2+} to the biomass which was immobilized within a polysilicate matrix. The binding of Ba^{2+} to that substrate displayed relatively constant binding for solution pH values of 2 to 6. Coincidentally, the color of the cell material was observed to reversibly darken with increased solution pH.

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