Pollution of the environment with toxic metals is widespread and often involves large volumes of wastewater. Remediation strategies must be designed to support high throughput while keeping costs to a minimum. Biosorption is presented as an alternative to traditional physicochemical means for removing toxic metals from wastewater. We have investigated the metal binding qualities of two biomass byproducts that are commercially available in quantity and at low cost, namely “spillage”, a dried yeast and plant mixture from the production of ethanol from corn, and ground corn cobs used in animal feeds. The biomass materials effectively removed toxic metals, such as Cu, Cs, Mo, Ni, Pb, and Zn, even in the presence of competing metals likely to be found in sulfide mine tailing ponds. The effectiveness of these biosorbents was demonstrated using samples from the Berkeley Pit in Montana. Investigations included column chromatography and slurry systems, and linear distribution coefficients are presented. X-ray spectroscopy was used to identify the binding sites for metals adsorbed to the spillage material. The results of our experiments demonstrate that the biosorption of metals from wastewaters using biomass byproducts is a viable and cost-effective technology that should be included in process evaluations.

Introduction

Anthropogenic release of trace metals creates public health problems due to their toxicity and persistence in the environment. There are many sources of metal pollution including manufacturing processes, smelting and refining, electricity generation, agricultural fertilization, refuse and wastewater treatment, and fuel combustion (1). The metals released contaminate the atmosphere, soils, and aquatic ecosystems. The pollution is widespread, and it is generally children that are most exposed and susceptible to their toxic effects.

Mining operations are responsible for the contamination of soils and waters with tailings that release toxic metals such as As, Cu, Mo, Ni, Pb, and Zn. The tailings are often held in large stabilization ponds that can cover many acres. For example, the Berkeley Pit, part of the Butte Mine Flooding Superfund Site in Montana, covers 3.2 km$^2$ and is nearly 300 m deep. The result of an abandoned Cu sulfide mine, the metal-contaminated waters continue to rise and threaten the groundwater supply of the Butte metropolitan area. Removal of metals from such a large volume of acid mine drainage will require an inexpensive (on a volume basis) remediation strategy.

Conventional technologies for the removal of metals from contaminated waters rely on relatively expensive mineral adsorbents or chemical flocculating agents (2–4). Biosorption technologies in which living or dead biomass is used to accumulate heavy metals is a method that can replace conventional processes for remediating metal pollution in wastewaters (5, 6). Microorganisms including bacteria and algae have been investigated for their metal-binding characteristics in systems that include dead microbial biomass (7, 8) or living cells that may be immobilized in a gel matrix (9, 10). The major drawback of these microbial systems is the cost of growing a sufficient quantity of bacterial or algal biomass.

The removal of dissolved metals by plant tissues has been examined using a variety of biomasses that represent byproducts from other commercial processes. Decaying leaves of Plantanus orientalis (Plane tree) can remove Cr and Ni from solution (11). The root biomass of tobacco and tomato plants has been shown to absorb Sr from solution (12). Trace concentrations of Pb and Zn were successfully removed from solution by processed solid residue of olive mill products (13). In a similar fashion, the residues from an apple juice processing factory have been shown to bind a number of heavy metals from aqueous solution (14). A recent paper examined the binding of Cr to oat byproducts (15). These latter systems used waste biomass materials that have little commercial value and are good candidates for the development of inexpensive biosorption processes.

A significant body of work exists on the absorption of metals by fungal biomass (16). Bioaccumulation has been observed in living cells of Saccharomyces cerevisiae (17, 18). Good metal binding properties were observed using dead biomass from the molds Aspergillus niger (19–21), Rhizopus arrhizus (22), and Mucor sp. (19, 23). An interesting recent study used extended X-ray absorption fine structure (EXAFS) spectroscopy measurements to define the binding sites for Pb and Zn in the cell walls of Penicillium chrysogenum (24). It should be noted that fungal biomasses are often waste products of food, beverage, or pharmaceutical production and again represent good candidates for the development of inexpensive biosorption processes.

The current study measures the biosorption of metals using two commercially available waste biomass materials. The dried byproduct (called spillage) from the production of ethanol by the yeast fermentation of corn is sold at low cost ($70–80 per ton) as an animal feed ingredient. Ground corn cobs are a low-value ($100–125 per ton) feed ingredient mainly used in animal feeds as a bulking agent. These biomasses can be obtained by the ton at prices low enough...
to make them viable alternatives for the binding of metals from wastewaters. The current study investigates the binding of As, Cu, Mo, Ni, Pb, and Zn as nitrate salt solutions and within mixtures of artificial and actual mine wastewaters using column and slurry methods. In addition to these elements, Cs was studied to extend this research into the area of radionuclide remediation. Wastewaters generated by the production of fissionable materials at the Hanford site and others are rich in Cs that often represents the majority of the radioactivity in these wastewaters.

**Experimental Section**

**Preparation of Metal Solutions.** Metal solutions of the appropriate concentrations were prepared from 500 ppm analytical standards (Inorganic Ventures Inc., Lakewood, NJ) in type I deionized water as nitric acid mixtures. Ultrapure 16N HNO$_3$ was prepared through a double distillation process in-house and used as the stock for these experiments. A synthetic mine wastewater solution was designed to simulate the concentrations and pH of nonheavy metals in wastewater from a Canadian Cu mine (Table 2; J. K. Rigby, Jr., personal communication). Another type of synthetic mine wastewater was modeled after wastewater samples from the Berkeley Pit in Montana (25). The metals of interest (i.e., As, Cs, Cu, Mo, Ni, Pb, and Zn) were then added to these background solutions. The pH of all of the metal solutions was brought to approximately 3.5 using ultrapure nitric acid. The concentrations of metals in the final solutions were determined by inductively coupled plasma-mass spectroscopy (ICP-MS).

**Preparation of Biomass.** Spillage is a byproduct from the commercial production of ethanol through the brewers’ yeast fermentation of corn. The material is dried to 10% moisture at 200°C. Spillage was obtained from New Energy Company of Indiana (South Bend, IN). It is a mixture of yeast and other elements, Cs was studied to extend this research into the area of radionuclide remediation. Wastewaters generated by the production of fissionable materials at the Hanford site and others are rich in Cs that often represents the majority of the radioactivity in these wastewaters.

**Column Chromatography.** Simple columns were constructed using the barrel of a 60-mL syringe fitted with a valve on the tip. A small amount of glass wool was packed into the bottom of the syringe, and the biomass was added. Spillage material could be washed easily in the syringe column and was tamped down with the syringe piston following the first and second 250-mL aliquots of distilled water wash. The corn cob material was presoaked in water before packing into the syringe column. The system was run as an open chromato graphic column at a flow rate of 1 mL min$^{-1}$. An aliquot of metal solution (generally 60 mL) was added to the
TABLE 2. Composition of Wastewater Mixtures

<table>
<thead>
<tr>
<th>sample</th>
<th>Al</th>
<th>As</th>
<th>Ca</th>
<th>Cs</th>
<th>Cu</th>
<th>Fe</th>
<th>Mg</th>
<th>Mn</th>
<th>Mo</th>
<th>Na</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canadian wastewater</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>770</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>synthetic wastewater</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>heavy metals mix</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Montana mix</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>500</td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>Berkeley pit</td>
<td>224</td>
<td>8</td>
<td>379</td>
<td>2</td>
<td>241</td>
<td>466</td>
<td>532</td>
<td>216</td>
<td>6</td>
<td>499</td>
<td>1029</td>
<td>10</td>
<td>667</td>
</tr>
</tbody>
</table>

For the experiments performed at the MRCAT beamline, the undulator gap was tapered (ΔE ~ 1 keV) and scanned, while a double crystal silicon (111) monochromator was used to collect the X-ray energy. Higher order harmonics were removed with a harmonic rejection mirror. The I0 ion chamber was filled with a nitrogen/helium (10/90) gas mixture. The transmission and fluorescence ion chambers were filled with free-flowing Ar at atmospheric pressure. Linearity tests (28) were performed on all samples and indicated less than 0.3% nonlinearity in the experimental setup for a 50% attenuation of the incident X-ray beam intensity. All measurements were repeated at least three times, and the resulting χ(k) data were averaged.

For the experiments performed at the NIST beamline, a double crystal silicon (220) monochromator was used to collect the X-ray energy. No harmonic rejection was used for these experiments. The I0 ion chamber was filled with free-flowing nitrogen at atmospheric pressure. The transmission and fluorescence ion chambers were filled with free-flowing Ar at atmospheric pressure. Linearity tests (28) were performed on all samples and indicated less than 0.5% nonlinearity in the experimental setup for a 50% attenuation of the incident X-ray beam intensity. All measurements were repeated at least three times, and the resulting χ(k) data were averaged.

Due to the relatively low concentration of Zn and Pb in the biomass samples, and hence the low signal-to-noise ratio of the XAFS signal, measurements were repeated 30 and 60 times for the Zn and Pb samples, respectively, and the data were merged. MacXAFS analytical software (v4.0) was used in this study.

The XAFS data were subjected to established analysis procedures (30) which included the normalization of the extended fine structure to the absorption edge step energy and height, removal of a nonoscillatory background curvature, conversion to photoelectron wave vector (k) space, and Fourier transformation to radial coordinates. In this later form, the data can be interpreted similarly to a radial distribution function where the centroid of the Fourier peak corresponds to the average bond distance (uncorrected for an electron phase shift) and where the amplitude and width of the peak corresponds to the coordination and atomic disorder of the atomic shells contributing to the peak. To obtain quantitative measurement of the average near-neighbor environment around each constituent element (Cu, Pb, or Zn), the nearest neighbor Fourier peak, appearing between 1 and 2 Å, was Fourier-filtered to k-space and fitted using parametrized theoretical EXAFS spectra generated using the FEFF codes (v7.0) of Zabinski et al. (31).

Results and Discussion

Metal Binding by Biomass Column Chromatography. In the first series of column chromatography experiments, metals were applied individually as aqueous solutions at a concentration of 5 ppm. More than 50 mL of the 50 ppm solutions were applied to the columns during each run. For this set of experiments, only one metal was applied to each column. Eluate fractions were collected during the application of the metal solutions, and the concentration of metals exiting
the columns was determined by ICP-MS. The data have been corrected for the void volume water of approximately 5 mL and 8 mL for the spillage and ground corn cobs columns, respectively. Spillage material was effective in retaining most of the target metals when applied as single-metal aqueous solutions (Table 1). The concentration of metals eluting from the column after the application of 50 mL of the 50 ppm solutions (2.25 and 2.10 mg metal for spillage and corn cobs columns, respectively) was less than 5 ppm for Cu, Mo, Ni, Pb, and Zn. Arsenic and Cs were more poorly retained by spillage material. Ground corn cobs also were effective in retaining most of the metals applied to the columns (Table 1). The eluate fractions contained less than 0.05 ppm of the metals, with the exception of As and Mo which were not retained as well. The applied Cs was completely adsorbed to the ground corn cobs but not to the spillage. In contrast, Mo was more poorly retained on the ground corn cobs but adsorbed well to the spillage material.

A breakthrough curve from the chromatography of Pb on the spillage material is shown in Figure 1. In this experiment, Pb was continuously applied as a 500 ppm aqueous solution to 20 g of spillage. Overloading of the biomass occurred after the application of approximately 100 mL of the metal solution. Lead in the applied solution passed through the column without additional retention after approximately 200 mL had been applied (Figure 1). Of the 97.5 mg of Pb applied before this plateau was reached, 58.2 mg were bound to the biomass matrix (20 g).

A synthetic mine wastewater was made that included the major nontoxic elements found in wastewater from a Canadian Cu mine (Table 2). The synthetic mine wastewater was used as a background solution to which individual target metals were added at a concentration of 50 ppm. Only one target metal was passed through each column, and eluate fractions were tested for metals during the application of more than 50 mL of the 50 ppm solutions (2.25 and 2.10 mg metal for spillage and corn cobs columns, respectively). The background of competing ions clearly influenced the binding of target metals to spillage material (Table 1). Arsenic and Cs were still the most poorly retained. Copper, Mo, and Pb were still very well retained. The levels of Ni and Zn passing through the columns were greater in the presence of the synthetic mine wastewater than in the single metal solutions. However, more Mo was adsorbed to ground corn cobs in a synthetic mine wastewater background than in a single metal wastewater solution. The opposite effect was observed with spillage material.

A mixed metal solution was prepared containing As, Cu, Mo, Ni, Pb, and Zn, each at 50 ppm (Table 2). When more than 50 mL of the mixed target metal solution (2.25 and 2.10 mg of each metal for spillage and corn cob columns, respectively) was applied to spillage columns, Cu, Pb, and Mo were all well retained. Although Ni and Zn were well retained when applied in single solutions, their adsorption was reduced in the metal mixture, and breakthrough was observed after the passage of approximately 35 mL of the 50 ppm solution (data not shown). Similar experiments with ground corn cobs showed that Cu, Mo, and Pb were completely retained. Nickel and Zn also were well retained. Again, Mo binds better in the presence of other ions than in their absence.

A second synthetic mine wastewater was generated based on the concentration of metals reported for the Berkeley Pit in Montana (Table 2 (24)). Note that Cu, Mn, and Zn were in great excess relative to As and Pb. As more than 50 mL of this mixed metal solution was applied to a spillage column, Cu, Mn and Zn exhibited breakthrough after substantial binding had occurred. Arsenic was not well retained (77% of applied). Despite the binding of large amounts of Cu and Zn, Pb was still retained well (99% of applied) by the spillage. Similar results were obtained using ground corn cobs (Table 1). Again, Pb was well retained (96% of applied) even in the presence of a great excess of other metals and the adsorption of large amounts of Cu and Zn.

Samples of actual mine wastewaters were used from the Berkeley Pit in Montana, a copper sulfide mine. Data are presented here from surface water samples (Table 1), but samples from 200 m below the surface had similar metal contents and gave similar biosorption results. Although the background of competing metals was high (Table 2), Pb was very well retained by both spillage and corn cob biomass (96 and 90% retention, respectively), similar to the results obtained with synthetic waste mixtures. The retention of As from the Berkeley Pit wastewater was lower than that of Pb (45 and 69% retention for spillage and corn cob biomass, respectively). Nickel concentrations were as high as 0.1% in the wastewater, and substantial amounts were retained by both the spillage and corn cob biomass before breakthrough. The results obtained using mine wastewaters from the Berkeley Pit are encouraging and suggest that these biosorption matrices will have practical value for the development of remediation strategies.

**Slurry Experiments.** The binding of metals to biomass matrices was measured by following the removal of metals from solution in aqueous slurries. In simple stirred systems at room temperature, 10 g of matrix was exposed to 250 mL of 500 ppm target metal solutions in the synthetic mine wastewater background. These exposure levels are much higher than for the column chromatography experiments and greater binding was expected. Samples of the solution were analyzed after 1, 24, 48, and 72 h of exposure. After 72 h, the biomass was rinsed with deionized water by filtration, and then desorption into 250 mL of deionized water was followed for 72 h. The amount of Cu, Mo, and Pb bound to each matrix on a weight basis is given in Table 3. The highest value is for the binding of Pb to spillage material, reaching nearly 1% on a weight basis. In all cases, binding was rapid, with the vast majority of metal binding occurring during the first hour of exposure. Insignificant amounts of metals desorbed from the matrix over the additional 72 h in deionized water. The column breakthrough experiment in Figure 1 exhibited Pb binding of 0.3% on a weight basis. The difference may be due to the high rate of elution (short residence time) or to channeling (incomplete mixing) within the column.

**FIGURE 1. Breakthrough of Pb on columns of spillage material.** Pb was applied to the column at 500 ppm with a flow rate of 1 mL min⁻¹, and 10-mL eluate fractions were obtained and then assayed by ICP-MS. Duplicate experiments gave similar results.
TABLE 3. Metal Binding by Biomass in Slurries

<table>
<thead>
<tr>
<th>matrix</th>
<th>metal</th>
<th>( \mu g ) metal/g biomass</th>
<th>( % ) w/w</th>
<th>percentage bound in first hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>corn cobs</td>
<td>Cu</td>
<td>1336</td>
<td>0.13</td>
<td>90</td>
</tr>
<tr>
<td>corn cobs</td>
<td>Mo</td>
<td>1603</td>
<td>0.16</td>
<td>100</td>
</tr>
<tr>
<td>corn cobs</td>
<td>Pb</td>
<td>9934</td>
<td>0.99</td>
<td>85</td>
</tr>
<tr>
<td>spillage</td>
<td>Cu</td>
<td>928</td>
<td>0.09</td>
<td>100</td>
</tr>
<tr>
<td>spillage</td>
<td>Mo</td>
<td>4200</td>
<td>0.42</td>
<td>68</td>
</tr>
<tr>
<td>spillage</td>
<td>Pb</td>
<td>5260</td>
<td>0.33</td>
<td>92</td>
</tr>
</tbody>
</table>

A series of slurry experiments was conducted with different concentrations of Mo or Pb in aqueous solution. Slurries containing 10 g of matrix were exposed to 250 mL of 50, 100, 250, and 500 ppm solutions of target metals for 72 h while measuring the disappearance of metals from solution aliquots by ICP-MS. Adsorption isotherms were examined, and it was found that equilibrium conditions were not obtained even at the highest metal concentration used. Linear distribution coefficients \( K_d \) are reported as the slope of the line plotting \( \mu g \) metals absorbed per g biomass against \( \mu g \) of metal remaining per L of solution. For the spillage material, the \( K_d \)s were determined to be 7.9 and 13.9 L g\(^{-1}\) for Pb and Mo, respectively. For ground corn cobs, the \( K_d \)s were determined to be 9.1 and 10.2 L g\(^{-1}\) for Pb and Mo, respectively.

Nature of Metal Binding. The Cu K-edge X-ray absorption near edge structure (XANES) \( 2^{nd} \) of a spillage sample exposed to Cu solution was qualitatively compared to the Cu K-edge XANES of copper oxide and copper sulfide. These data indicate that the average stereochemical interaction between the Cu ions and the biomass are more similar to copper sulfide interactions than to copper oxide interactions (data not shown). Examination of the Cu EXAFS illustrated the quality of the Cu EXAFS dataset and also indicated the average local chemical environment of the Cu in the biomass to be consistent with the Cu ions bound to sulfur in the same fashion as that of copper sulfide (data not shown). Finally, Figure 2A is a representation of the Fourier transforms \( (\Delta K = 2.0-9.0 \text{ Å}^{-1}, \Delta k = 1.0 \text{ Å}^{-1}) \) of the \( k^2 \)-weighted data of the Cu EXAFS. A closer inspection of these data also indicates direct interaction between the Cu ions and sulfur atoms. Additionally, inspection of the higher order coordination shells in these Fourier transforms \( (R = 2.5-4.0 \text{ Å}) \) indicates that the Cu was not present in the biomass sample as a crystalline form of CuS. Results of fitting analyses could not definitively identify an atomic species beyond the first coordination shell of sulfur.

The results of Zn K-edge XAFS obtained from a zinc oxide powder standard and spillage biomass exposed to a Zn solution are shown in Figure 2B. In contrast to the results obtained from the Cu XAFS study, the Zn XAFS results indicate that the primary interaction between Zn ions and biomass was via an oxygen atom. To quantify the average coordination environment around the Zn ions, the Fourier transformed data (Figure 2B) were Fourier filtered \( (R_{\text{min}} = 0.45 \text{ Å}, R_{\text{max}} = 2.30 \text{ Å}) \). Results of a fit of that data with similarly filtered data from the zinc oxide standard indicated an average Zn–O coordination number of 5 ± 1, an average Zn–O radial distance of 2.10 ± 0.015 Å, and a change in the XAFS Debye–Waller factor (relative to the zinc oxide standard) of −0.003 ± 0.002 Å\(^2\). Although fits to the Zn XAFS data with Zn–S correlations were attempted, no statistically significant fits to the data were achieved.

The results of Pb L-III-edge XAFS obtained from a lead oxide (PbO) powder standard and spillage biomass exposed to a Pb solution are shown in Figure 2C. Similarly to the results obtained from the Zn XAFS study, the Pb XAFS measurements indicate that the primary interaction between Pb ions and biomass was through an oxygen atom. However, inspection of the Fourier transform of the data (Figure 2C) indicates the presence of a “shoulder” on the right side of the first Fourier transform peak. This is indicative of an additional back-scattering atom at a radial distance greater than the Pb–O radial distance. To quantify the average coordination environment around the Pb ions in the biomass, the Fourier transformed data (Figure 2C) were Fourier filtered \( (R_{\text{min}} = 0.75 \text{ Å}, R_{\text{max}} = 3.2 \text{ Å}) \). Results of a fit of that data with theoretical...
standards generated by the FEFF codes (v7.0 (30)) indicated the primary signal to be due to a Pb–O correlation. Two different fitting scenarios were used in an attempt to fit the higher coordination shell: (1) to simulate a carboxyl-type of bonding between the biomass and the lead ions, a carbon atom was used to fit the higher coordination shell, and (2) to simulate the presence of an additional Pb–S interaction between the biomass and the lead ions, similar to that seen in the Cu K-edge XAFS results, a sulfur atom was used to fit the higher coordination shell. Unfortunately, due to the limited k-range of useful data available for Fourier transformation (3.0–9.0 Å⁻¹), equally good fits to the data were achieved by assigning the identity of the higher coordination shell to be either sulfur or carbon.

Given the complexities of plant and fungal cell walls, there are many functional groups available for metal binding including carboxyl, hydroxyl, phosphoryl, and sulfhydryl groups. It has been reported (24) that fungal cell walls bind Pb and Zn predominantly through phosphoryl moieties with some binding to carboxyl groups (especially strong at low Pb concentrations). In agreement, the current report demonstrates Pb and Zn binding to oxygen atoms.

The current study has demonstrated the biosorption of metals from actual and synthetic wastewaters by two agricultural byproducts; spillage, a yeast-plant biomass remaining after the manufacture of ethanol from corn, and ground corn cobs from the production of animal feeds. Given the specific binding abilities demonstrated here, there is excellent potential to remove toxic heavy metals from wastewaters using these biomasses, with several advantages desirable for wastewater treatment schemes. The biomass is combustible, unlike many competing inorganic matrices. Thus, after binding metals, the biomass can be properly incinerated to greatly reduce its bulk volume and thereby dramatically reduce stabilization costs. The biomass byproducts are readily available by the ton from sources nationwide at low cost. Given the realities of wastewater remediation projects, low-cost, high-volume processes are a necessity. The results of our experiments demonstrate that the biosorption of metals from wastewaters using biomass byproducts is a viable and cost-effective technology that should be included in process evaluations.

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