Uptake of Metal Ions by *Rhizopus arrhizus* Biomass

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Rhizopus arrhizus biomass was found to absorb a variety of different metal cations and anions but did not absorb alkali metal ions. The amount of uptake of the cations was directly related to ionic radii of La^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}, Hg^{2+}, Pb^{2+}, UO_2^{2+}, and Ag^+. The uptake of all the cations is consistent with absorption of the metals by sites in the biomass containing phosphate, carboxylate, and other functional groups. The uptake of the molybdate and vanadate anions was strongly pH dependent, and it is proposed that the uptake mechanism involves electrostatic attraction to positively charged functional groups.

It is well documented that microbial biomass is capable of absorbing metal ions from aqueous solutions even when the cells have been killed (5, 6, 12, 18, 25, 26). It has been proposed that these materials could be used to decontaminate waste streams from mining, refining, nuclear fuel processing, electroplating, and other industries and to concentrate metals (14, 25, 26, 30). Cells of *Saccharomyces cerevisiae*, *Pseudomonas aeruginosa*, *Actinomyces levoris*, *Streptomyces viridochromogenes*, *Rhizopus arrhizus*, and many other organisms have all been shown to accumulate uranyl ions (14, 24–26). The *R. arrhizus* biomass was the most efficient, absorbing up to 180 mg of uranium per g of biomass (26). In the present study, the ability of *R. arrhizus* cells to absorb other metal ions is examined.

Studies on the uptake of metals by biomass have been complicated by the nature of both the absorbent and the metal species in aqueous solution (3, 6). The cell walls of *R. arrhizus*, or any microbe, contain many potential sites for the uptake of ions (2, 5, 11), and it is unlikely that any one type of molecule or functional group is responsible for the absorption of the metals. Many of the metals have complex solution chemistries (1, 4, 27, 28), and it is not always possible to determine what species are actually present. The equilibria involved are dependent on pH, concentration, the anions present, and other factors.

The uptake of metals by the chemically simpler ion-exchange resins is usually assumed to be due to mainly electrostatic attraction. In homologous series of ions, such as the alkali metal ions or the La^{3+} ions, correlations were found between the radius of the hydrated ions and uptake (8, 17). There is a roughly inverse correlation between ionic radius and hydrated radius for these ions, and uptake increases as ionic radius increased (17, 29).

The purpose of this research was to test *R. arrhizus* biomass for the ability to absorb metal ions other than uranyl, to attempt to correlate the degree of uptake with properties of the metal ions, and to study the nature of the sites in the biomass which absorb the ions.

MATERIALS AND METHODS

Preparation of biomass. The *R. arrhizus* strain used was obtained from Canada Packers Ltd., Toronto, Ontario (strain no. 126948-79). The growth medium used contained peptone (0.5%), neopeptone (0.5%), sucrose (2%), KH_2PO_4

(0.1%), $NaNO_3$ (0.1%), and $MgSO_4 \cdot 7H_2O$ (0.05%). The microorganism was maintained on solid medium obtained by adding 2% agar to the above medium. Cells for the uptake studies were obtained by culturing in liquid medium at 25°C for ca. 70 h.

The cells were separated from the broth by filtration, washed with deionized distilled water, resuspended, and washed again. The biomass was dried for 10 h at 60°C under reduced pressure. The product was ground in a mortar and pestle.

Metal uptake experiments. The ions studied included the nitrate salts of Cr(III), La(III), Mn(II), Cu(II), Zn(II), Cd(II), Ba(II), Pb(II), Ag(I), Na, K, Rb, and Cs as well as uranyl nitrate, mercuric chloride, sodium vanadate, and sodium molybdate. For each isotherm, series of flasks were prepared with known volumes of serial dilutions of standardized metal salt solutions. The pH was adjusted with 1 N HNO_3 or 1 N NAOH. Weighed quantities of the dried biomass were added, and the flasks were agitated at 200 rpm at 25°C for 18 h. The biomass was removed by filtration through a 0.45 μm Millipore membrane filter, and the filtrates were analyzed for metal concentrations.

A Perkin Elmer model 403 atomic absorption spectrophotometer was used to determine Cr, Mn, Cu, Zn, Cd, Ba, Pb, Ag, Na, K, Rb, and Cs. Uranyl and La^{3+} ions were determined by the method of Savin (16, 21). The standard peroxide and thiocyanate colorimetric methods were used to assay for vanadate and molybdate (16, 23). Mercury(II) was determined by the standard dithizone technique (16, 23). These data were used to calculate the number of moles of metal ion absorbed per gram of biomass.

RESULTS

Absorption studies were conducted with ionic species of 17 different metals with widely different behavior in aqueous solution. Uptake data for each ion were plotted against the final metal concentration (C_f) in the solution. Representative isotherms for UO_2^{2+}, Ag^+, and Cu^{2+} are plotted in Fig. 1. At least three sets of data were obtained for each ion. Control experiments with no biomass were done for each metal ion. The metal ions were not absorbed by the filter membrane.

The maximum uptake (q_L) was determined by fitting the data to the Langmuir absorption model (equation 1) (15) with a nonlinear regression analysis (20): $q = q_L(bC_f)/(1 + bC_f)$. The resulting values of q_L are listed in Table 1 for the cationic species at an initial pH of 4. Also included are the

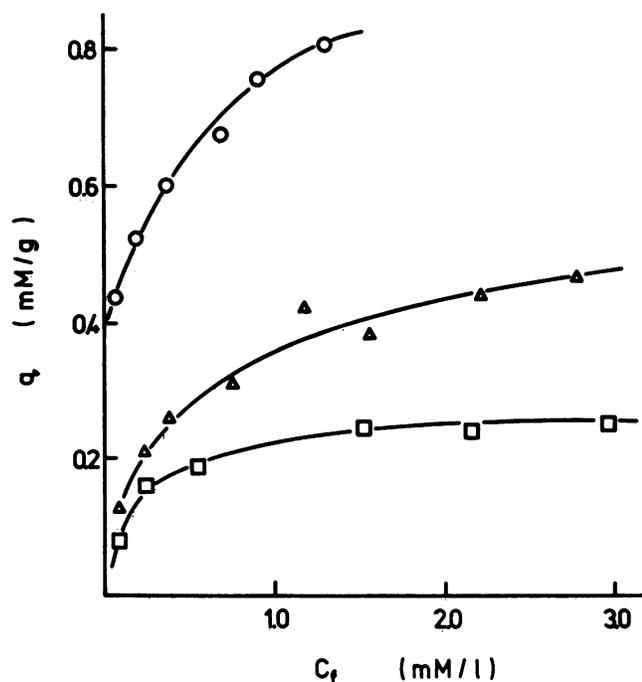FIG. 1. Representative absorption isotherms at 25°C and ca. pH 4. Symbols: $\bigcirc$, UO_2^{2+}; $\triangle$, Ag^+; and $\square$, Cu^{2+}.

final pH values at maximum uptake. In general the variation in pH is relatively small. Buffers could not be used because they all had the ability to complex many of the metal ions to some degree, and preliminary studies showed that this interfered with uptake.

All of the cations were absorbed with the exception of the four alkali metal ions. The highest uptake, of 0.82 mM/g was observed for UO_2^{2+}.

Maximum uptake of these cations is plotted against estimated ionic radius (10, 19) in Fig. 2. With the exception of Cr^{3+}, the data for the ions which are absorbed all lie on a straight line, illustrating increasing uptake with increasing ionic radius. No correlation was observed with ionic charge or effective nuclear charge of the ions.

Uptake was studied with various oxy-ions. At pH 3.5, uranyl uptake was 0.82 mM/g, but at pH 2.5 it was only 0.30

TABLE 1. Uptake of metal cations by *R. arrhizus* biomass

Metal ion	g_L (mM/liter)	Confidence interval	Final pH
Cr^{3+}	0.59	0.09	3.7
La^{3+}	0.35	0.04	3.6
Mn^{2+}	0.22	0.04	4.2
Cu^{2+}	0.25	0.02	3.5
Zn^{2+}	0.30	0.06	4.0
Cd^{2+}	0.27	0.05	4.0
Ba^{2+}	0.41	0.07	4.0
Hg^{2+}	0.29	0.12	3.5
Pb^{2+}	0.50	0.04	3.6
UO_2^{2+}	0.82	0.08	3.5
Na^+	0.0	0.05	4.4
K^+	0.0	0.05	4.3
Rb^+	0.0	0.05	4.6
Ag^+	0.50	0.06	4.2
Cs^+	0.0	0.05	4.6

mM/g. At pH 4.5, the q_L values of MoO_4^{2-} and VO_3 were 0.38 and 0.45 mM/g, respectively, but at pH 5.5 neither anion was absorbed and the q_L values were 0.0. It was not possible to obtain data for each ion over a wider range of pH because of the limitations imposed by the solution chemistry of the ions.

DISCUSSION

To facilitate a comparison of the results, most of the data in the present study were obtained with a single counterion, nitrate, and a pH as close as possible to 4. This pH was chosen to avoid the precipitation of metal oxides (1). Nitrate salts are usually soluble, and the nitrate ion forms only weak complexes with metals (9, 16). To avoid complexation of the metals by other anions, all the data are reported for unbuffered solutions. Table 1 records the final pH. In each case this is an average of several values. The variation in final pH was typically less than 0.5 pH units, but this did not affect the q_L values. Thus it seems reasonable to assume that the variation in final pH among the various ions does not significantly alter the correlations.

The Langmuir parameter, q_L, corresponds to the maximum uptake of a metal ion by the *R. arrhizus* biomass (15). Figure 2 shows the relationship between q_L and the ionic radii of various cations. Most of these ions were absorbed strongly by the *R. arrhizus* biomass. In general, the data showed a linear correlation between maximum uptake and ionic radius, except for Cr^{3+}, and the alkali metal ions. There was no correlation with effective nuclear charge on the ions or with formal charge.

At pH 4 there would be few functional groups in the biomass with negative charges. It is proposed that a significant portion of the uptake is due to coordination of functional groups on molecules in the biomass to the metal ions. The alkali metal ions (Na^+, K^+, Rb^+, and Cs^+) were not absorbed by the biomass. This group of metals does not form complexes with most ligands. The absence of absorption of these ions is consistent with the proposed uptake mechanism of the nonalkali ions by the biomass, involving binding by functional groups.
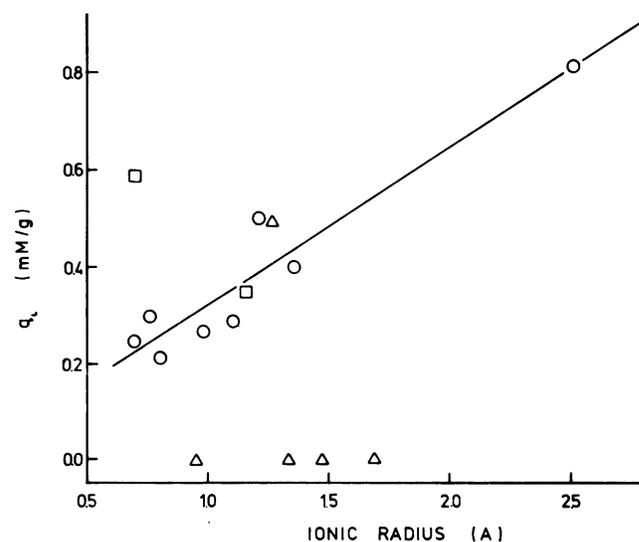

FIG. 2. q_L of cationic species plotted against ionic radius. Symbols: $\triangle$, ionic charge + 1; $\bigcirc$, ionic charge + 2; and $\square$, ionic charge + 3.

It is difficult to establish at this point the nature of the site of uptake because there are many potential "ligands," including carboxylate, amine, phosphate, hydroxyl, sulfhydryl, and other functional groups (2, 5, 11). Furthermore, any site could have several different functional groups participating to various degrees in binding the ions.

At pH 4 the primary amines would be positively charged and not expected to interact with cations. Many of the carboxylate groups would be neutral; however, the acidic protons could be displaced relatively easily by the metal ions. The uptake of the uranyl ion was lower at pH 2.5 than at pH 3.5, which is consistent with competition by protons for the sites. Most of the phosphate groups present as mono- and diesters would have a negative charge above pH 3 (7, 22) and could contribute appreciably to the binding. Hydroxyl and amide functional groups are very weak bases and could form only weak bonds with the metals. It is likely that most of the sites of metal uptake contain carboxylate or phosphate ligands or both. These primary bonds may be augmented by association with hydroxyl and other groups.

If the uptake is due to electrostatic attraction of the solvated ions, then the most important groups at pH 4 would be the phosphates. These would have a negative charge under these conditions. The trend in uptake in Fig. 2 would be consistent with this mechanism except for the results with the alkali ions. Other studies where electrostatic attraction is important show appreciable absorption of these ions (8, 17, 31).

Chromium(III) was absorbed at anomalously high levels. Chromium in aqueous solution forms various complex oxyanions (9). The actual charge of the complexes is <3, and the actual radii are significantly larger than that for a simple chromium(III) ion. Possibly the chromium data would fall on the line if the appropriate radius were known and the data were plotted accordingly.

Molybdenum and vanadium ions are hydrolyzed in water to various soluble species (1). These are mainly anionic above pH 4. Solutions of sodium vanadate or sodium molybdate both exhibited significant uptake by the biomass at pH 4.5, but neither system showed absorption at pH 5.5. At pH 4.5 many of the nitrogen-containing functional groups in the biomass would be quaternized, and the positive charges would attract the anions. Possibly at pH 5.5 the balance of positively and negatively charged species has become unfavorable for anion binding. Most of the common amino acids have isoelectric points in the range of pH 5 to pH 6. The *R. arrhizus* biomass appears to have an overall "isoelectric point" below pH 5.5.

A relationship between ionic size and absorption has been demonstrated previously for a much simpler system than biomass (8, 13, 17). Studies involving Dowex 50 ion-exchange resin show that uptake depended on ionic charge and could be correlated with ionic radius or hydrated radius. The alkali metal ions were also absorbed by the resin. In this system, the uptake mechanism includes electrostatic attraction since Dowex 50 is a strong acid ion-exchange resin.

The earlier work with *R. arrhizus* biomass involved two metals (27, 28) and demonstrates the importance of the solution chemistry of the metals when interpreting results. It was found with electron microscopy that the uranyl ion was absorbed into the biomass. Thorium(IV) was deposited only on the surface of the biomass. This is consistent with hydrolysis of thorium(IV) in water to form colloidal suspensions which are coagulated by the biomass.

A recent publication reported the competition of various metal ions with the uptake of uranyl by a purified biopoly-
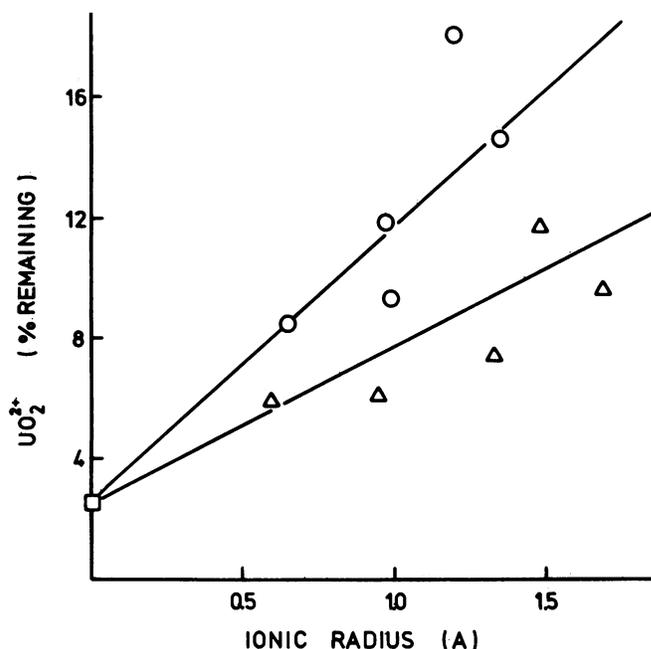

FIG. 3. Percentage of initial UO_2^{2+} remaining in solution plotted against the ionic radius of competing ions in uptake competition studies (31). Symbols: $\triangle$, ionic charge + 1; $\bigcirc$, ionic charge + 2; and $\square$, no competing ions.

mer, emulsan (31), at pH 7.2. Emulsan is an acidic polymer (32) and would be highly negatively charged at pH 7.2. We have plotted this data in Fig. 3. The result was similar to that observed for Dowex 50. Again there were two groups of metal ions—the alkali ions being absorbed to a lower degree than the divalent cations. However, there was significant absorption of the alkali ions, unlike the *R. arrhizus* biomass, and this indicates that the mechanisms of metal ion uptake are different.

R. arrhizus biomass absorbs a variety of metal ions at pH 4. The alkali ions are not absorbed, and larger ions are absorbed more strongly than smaller ions. This behavior can be explained by a complexation mechanism involving sites in the biomass containing carboxylate, phosphate, and other functional groups. The uptake studies with ion exchange resins and with emulsan both show strong absorption of alkali ions, and the mechanism is electrostatic attraction of the solvated ions.

The *R. arrhizus* biomass is unusual in that it does not absorb appreciable amounts of alkali ions. This is industrially significant because in many potential applications unwanted alkali ions will be present and in competition for uptake with the desired metal ions.

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