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BIOSORPTION OF METAL IONS USING CHITOSAN, CHITIN, AND BIOMASS OF *RHIZOPUS ORYZAE*

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ABSTRACT

The biosorptive capacity of dried biomass fungus *Rhizopus oryzae* Went & Prinsen-Geerlings for metal sorption was compared with commercially available sources of chitin, chitosan and chitosan cross-linked with benzoquinone. Initial pH of the metal solution significantly influenced metal uptake capacity. The optimum biomass/solution ratio for metal uptake in all systems was 1 g/L. The highest metal uptake values (137, 108, 58, and 124 mg/g, respectively, for copper, zinc, arsenic, and chromium) were achieved with chitosan (1 g/L, at pH 4) from initial metal concentrations of 400 mg/L. Decreases in mean metal concentrations from a simulated copper/zinc mine effluent were 73%, 14%, and 36% for copper, aluminum, and zinc, respectively, which corresponded to re-

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spective metal uptake values of 16, 11, and 21 mg/g. Sorption from a simulated gold mine effluent showed decreases in mean concentrations of aluminum, arsenic, and copper of 85%, 30%, and 92%, respectively, which corresponded to respective metal uptake values of 3.0, 6.0, and 1.6 mg/g. The observed decreases in copper levels to concentrations below 1 mg/L indicate potential for specific polishing applications. At low pH, *R oryzae* biomass was more resistant than was chitosan. Cross-linking with benzoquinone under alkaline conditions conferred stability to the chitosan biomass under low pH, but some reduction in sorption capacity was observed.

Key Words: Biosorption; Chitosan; Copper; Zinc; Chromium; *Rhizopus*

INTRODUCTION

The fate of heavy metals in the environment has become an increasing concern. In addition to toxic environmental effects, copper, chromium, and zinc have been associated with various chronic disorders in humans. Precipitation, oxidation and reduction, ion exchange, filtration, reverse osmosis, electrochemical removal, and evaporative recovery can all potentially be used to treat industrial effluents for metals. Biosorption, using either living or dead biomass, has been proposed as a potential alternative to chemical techniques for the removal and recovery of metal ions from aqueous solutions. Efficient metal biosorption with initial binding sometimes followed by precipitation of metal at up to 25% of the dry weight has been demonstrated with microbial biomass (1).

In addition to layers of glucans, glycoproteins, and proteins, fungal cell walls may contain chitin and/or chitosan. The cell walls of Mucorale fungi are characterized by a predominance of chitosan rather than the acetylated form, chitin (2). Chitin and chitosan do not appear to be the major metal-binding compounds active in the complex sequestering of metals by biosorption (1) although their involvement in the process, through formation of metal-nitrogen complexes in the amino groups, was described for the accumulation of uranyl ions via *Rhizopus arrhizus* (3,4). Work with seaweed biomass has indicated the involvement of acidic sulfate groups of fucoidan and carrageenan in heavy metal biosorption (5).

The biosorbent potential of filamentous fungi, several of which are by-products from the fermentation of metabolites in enzymes, flavorings, or antibiotics, has been studied. One of the most widely studied fungi is *R arrhizus* (3,6–9). Uptake of chromium (VI), cobalt, nickel, copper, zinc, cadmium, lead, thorium, and uranium through the use of *Rhizopus* species biomass has been reported (10).

Chitin, along with cellulose, is one of the most abundant and inexpensive forms of biomass. Chitosan is easily prepared from chitin by deacetylating the acetoamide groups with strong alkaline solution. Both chitin and chitosan are waste products from the seafood industry. Chitosan has been used to remove trace quantities of nickel from industrial effluents (11). Chitosan has also been used to absorb various organic substances, such as polychlorinated biphenyls, proteins, and nucleic acids. It has also been investigated for applications in separation, concentration, and purification of metals in the hydrometallurgy industry (12).

Chitosan has been derivatized with different functional groups to increase the specificity of the biopolymer for particular metal ions such as α-keto (13), ascorbic, and oxo-2-glutaric acids (14). Chelating agents such as ethylenediaminetetraacetic acid (15) and *N*-carboxymethyl derivatives of chitosan have also been synthesized (12,16). Although the mechanism of metal adsorption by chitosan has not been completely elucidated, the chitosan molecule has reactive amino and hydroxyl groups that, together with the flexible structure of the polymer chains of chitosan, result in a configuration favorable for complexation with metal ions (3,17).

Biological materials may have limitations, such as low metal-binding capacity, low specificity, and poor chemical and/or mechanical stability. Mechanical and chemical stability has been conferred by immobilization techniques, such as adsorption on inert supports, entrapment in polymeric matrices, covalent bonding to carriers, and chemical cross-linking (18,19).

This study systematically compares sorption parameters over ranges of pH (2–7), metal concentration (5–400 mg/L), and biomass loading (1–10 g/L) for copper (Cu^{2+}), zinc (Zn^{2+}), arsenic (AsO_4^{2-}), and chromium (CrO_4^{2-}) using dried biomass of *R oryzae*, chitosan, and chitin. A preliminary estimation of the potential for metal removal from simulated mine effluents through the use of chitosan is described.

EXPERIMENTAL

R oryzae Went & Prinsen-Geerlings (Zygomycetes, Mucorales, syn: *R arrhizus* Fischer) DAOM 148428 was grown in a liquid culture medium containing the following (in g/L) for 48 hours at 28°C at 100 rpm: peptone, 10; sucrose, 20; KH_2PO_4, 1; $NaNO_3$, 1; and $MgSO_4 \cdot 7H_2O$, 0.5. Cultures were filtered and the retained biomass was washed twice in deionized water and dried at 60°C overnight. Dried biomass was pulverized and stored in the presence of a desiccant at room temperature. Chitosan (high molecular weight, 75–85% deacetylation) and chitin were used as provided by the respective suppliers (Aldrich Chemical Co, Milwaukee, Wis, and Sigma Chemical Co, St Louis, Mo). The chitosan had the following particle size distribution: +3.36 mm, 6.2%; −3.36 mm +2.38 mm, 34.3%; −2.38 mm +1.68 mm, 29.1%; −1.68 mm +850 μm, 22.4%; −50 μm +595 μm, 3.8%; −595 μm +425 μm, 1.4%; −425 μm, 2.4%. The chitin had the following particle size distribution: +425 μm, 8.0%; −425 μm +300 μm, 33.5%; −300 μm +212 μm, 21.0%; −212 μm + 177 μm, 5.5%; −177 μm +106 μm, 5.5%; −150 μm +5.5%; −106 μm, 18.5%.

Biosorption was determined by contacting known quantities of biomass (1–10 g/L) with solutions of metal ions within a range of initial concentrations from 25–400 mg/L at initial pH values ranging from 2–7. Batch sorption experiments were carried out through the addition of a measured quantity of biomass to 100 mL of metal solution in a 250-mL Erlenmeyer flask. Metal- bearing solutions were prepared by dissolving the following metal salts (analytical grade) in deionized water: $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2HAsO_4 \cdot 7H_2O$, and K_2CrO_4. The pH was adjusted with HCl or NaOH as required. Biosorption was performed under agitation on an orbital shaker at 100 rpm at 25°C overnight. The equilibrium solutions were separated from the biomass by vacuum filtration through membrane filters (0.45 μm). The biomass retained on the filter was dried, weighed, and digested with nitric acid (20). Metal ion concentrations of the solutions at time 0, at equilibrium, and in the digested biomass were determined by an inductively coupled plasma atomic emission spectrometer (ICP-AES) with a Jarrell-Ash Atomscan 2400. Metal uptake (mg/g) was calculated from the difference in the metal contents in the aqueous solution (mg/L), before and after adsorption, and to the dry weight (g/L) of the biomass. Mass balance was confirmed by verification with the metal concentrations in the recovered biomass. Experimental values of metal uptake capacities are the results of triplicate experiments reported as means. Control tests without the addition of biomass were carried out for each biosorption series. After an initial series of sorption experiments was completed, a final experiment, in which the best combinations of factors for each metal were examined, was performed. Isotherms were determined on 3 types of biomass: chitin, stabilized chitosan, and *R oryzae* through a biomass/liquid ratio of 1 g/L, an initial aqueous metal concentration of 400 mg/L, and pH 5 for Cu and Zn and pH 2 for As and Cr.

The simulated mining effluents representative of effluent discharge samples from Canadian copper/zinc and gold mines (21) were prepared from the elements outlined in Table 1. Biosorption was conducted as described above using chitosan (1 g/L), at pH 4 and 25°C with effluent samples removed from solution at varying time intervals during a 24 hour period.

Chitosan (18 g in 400 mL of deionized water) was stabilized by a reaction with benzoquinone (5.4 g in 100 mL of deionized water) (Acros Organics N.V., Fairlawn, NJ) under alkaline conditions. The slurry was mixed for 15 minutes before NaOH (4 g in 100 mL deionized water) was added and the mixture was stirred for 60 minutes. The solid, resulting from filtration of the slurry, was washed extensively with deionized water and air-dried overnight at room temperature prior to use.

Table 1. Elemental Composition of Simulated Effluents

		Element Concentration (mg/L)	
Element	Salt	Copper/Zinc Effluent	Gold Effluent
Al	$AlK(SO_4)_2 \cdot 12H_2O$	16.5	4.6
Ca	$Ca(SO_4)_2 \cdot \frac{1}{2}H_2O$	97.0	155.0
Cu	$CuSO_4 \cdot 5H_2O$	24.0	1.0
Mg	$Mg(SO_4) \cdot 7H_2O$	0.0	46.0
Na	$Na_2(SO_4)$	53.0	112.0
Sr	$SrCl_2 \cdot 8H_2O$	2.0	13.8
Zn	$ZnSO_4 \cdot 7H_2O$	61.0	0.0

RESULTS AND DISCUSSION

Kinetic Studies

The physicochemical parameters examined were based on previous literature reports (18,19) and from preliminary kinetic studies performed with chitosan (1 g/L) at pH 4 and metal concentrations of 50 mg/L where liquid samples were removed and analyzed over time. Under the experimental conditions, with most of the metals examined, equilibrium conditions occurred within 2 hours (Fig. 1). A contact time of 16 hours, providing more than sufficient time for equilibrium conditions to be reached, was used in subsequent studies.

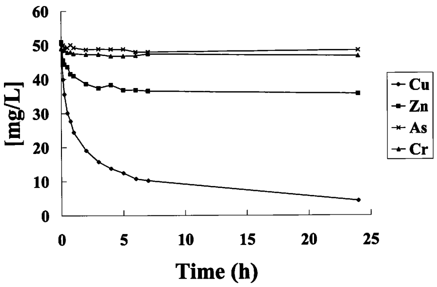

Figure 1. Chitosan sorption kinetics for copper, zinc, arsenic, and chromium at pH 4 and 25°C.

Effect of Initial pH

Batch experiments showed that pH had a major effect on biosorption (Figs. 2a, 3a). Chromium uptake (10.6 mg/g) occurred only at pH 2 with *R oryzae* (Fig. 2a). The dissolution of chitosan at pH 2 made completion of the corresponding experiments impossible. At pH 3, chromium uptake was 39 mg/g, with sorption rapidly dropping off with increasing pH (Fig. 3a). The optimum pH for

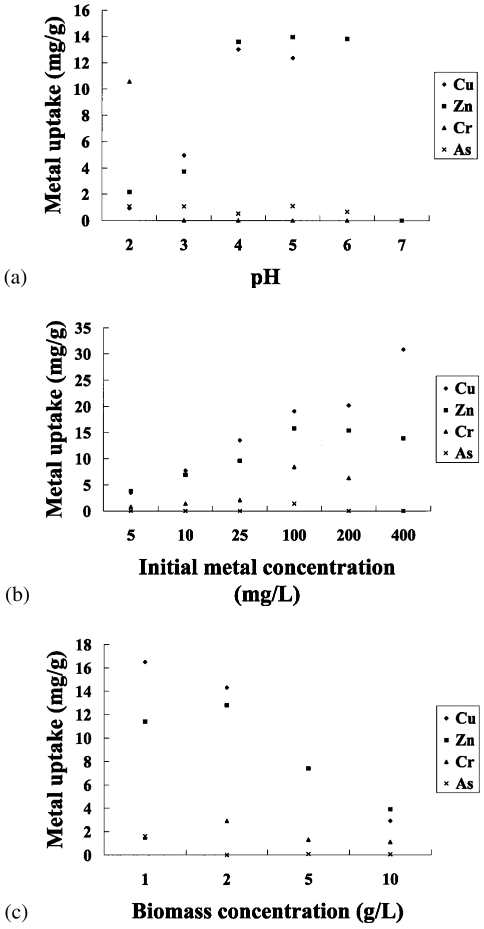

Figure 2. Sorption of Cu, Zn, Cr and As by *R oryzae*. (a) Effects of pH on metal uptake (mg/g) with initial metal and biomass concentrations at 50 mg/L 1 g/L respectively. (b) Effect of initial metal concentration on metal uptake (mg/g) at pH 4 and biomass concentrations 1 g/L. (c) Effect of biomass concentration on metal uptake (mg/g) at pH 4 and initial metal concentrations of 50 mg/L.

chromium biosorption reported in previous studies with peat moss (10), corn (22), and seaweed (23) ranged from 1.5–2.5. Literature reports from researchers who used the fungus *Mucor hiemalis* with CrO_4^{2-} (24), describe greatest chromium adsorption (4.3 mg/g) occurring at pH 1.

Limited sorption of arsenic with *R oryzae* was observed over the entire pH range examined (Fig. 2a). The uptake of this metal by chitosan was also low for most of the pH range, except at pH 3 (32 mg/g) (Fig. 3a). Previous reports of arsenic uptake include that of an algae-based arsenic removal system (25) and the BIO-FIX process composed of peat-based biomass immobilized in polysulfone (26). While researchers have reported (12) that chitosan was soluble in aqueous solutions of organic and inorganic acids except for sulfuric acid, we observed that chitosan dissolved rapidly in 1N H_2SO_4 and in aqueous metal solutions in which pH was adjusted to below 3 with H_2SO_4.

Values above pH 6 are not shown for zinc due to observed precipitation. Sorption was equal over a pH range from 4–6 with uptake of approximately 14 mg/g for both types of biomass.

As a result of copper precipitation above pH 5, results for pH 6 and 7 are not presented. Copper accumulation was greatest with both *R oryzae* and chitosan at pH 4 and 5 with respective uptakes of 12–13 mg/g and 41–42 mg/g. At pH 3, sorption of copper with chitosan was 30 mg/g while the corresponding value with the fungal biomass was less than 5 mg/g.

Similar trends with respect to pH have been observed by other researchers (6) for biosorption of Cr and Cu(II) ions by *R arrhizus* and for Cu and Zn ions (27). Adjustment of pH has been suggested as a tool for obtaining selective metal uptake (28). The amino functional group of chitosan is probably involved in biosorption of copper and zinc and also with the anions; at low pH values the amino functional groups of chitosan will be protonated and the biomass will thus behave as an anion exchanger. The increase in copper uptake with increasing pH is consistent with the greater stability of transition metal–amine complexes at high pH values.

Effect of Initial Metal Concentration

Metal uptake with chitosan was positively correlated with increasing metal concentrations (Fig. 3b). A similar effect was observed for copper with *R oryzae* biomass while Zn and Cr reached maximum uptake at concentrations of 100 mg/L (Fig. 2b). The highest metal uptake values were achieved with chitosan. They are respectively 137, 108, 58, and 124 mg/g for copper, zinc, arsenic, and chromium and correspond to total metal accumulations in chitosan (dry weight) of 8%, 7%, 4%, and 9%. The opposite effect has been observed with *Aspergillus niger* biomass in which uptake decreased with increases in initial concentrations of Cu and Zn ions (27).

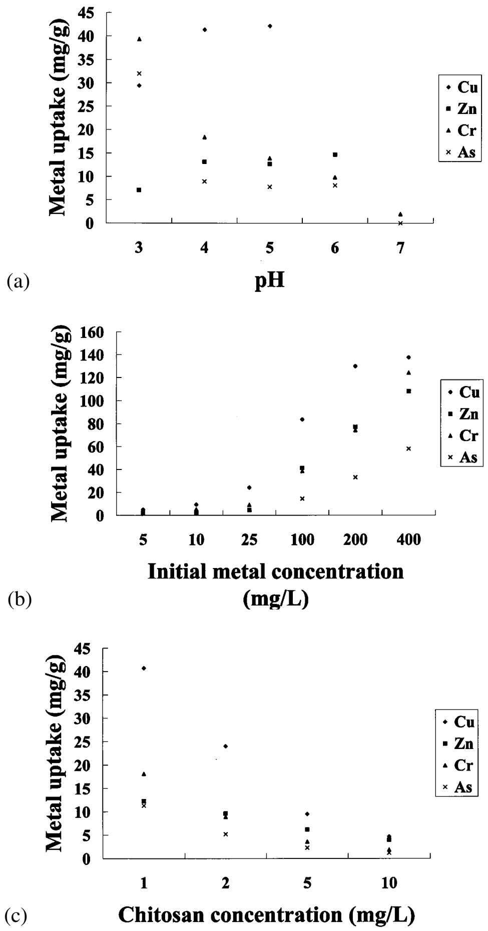

Figure 3. Sorption of Cu, Zn, Cr and As by chitosan. (a) Effects of pH on metal uptake (mg/g) with initial metal and biomass concentrations at 50 mg/L and 1 g/L respectively. (b) Effect of initial metal concentration on metal uptake (mg/g) at pH 4 and biomass concentrations 1 g/L. (c) Effect of biomass concentration on metal uptake (mg/g) at pH 4 and initial metal concentrations of 50 mg/L.

Effect of Biomass Loading

Biomass loading can influence the extent of metal uptake from solution (27). A biomass loading of 1 g/L resulted in the greatest metal uptake with both the fungal biomass and the unmodified chitosan for all metal ions tested (Figs. 2c, 3c). Similar to reports in the literature (27), increased metal uptake was observed with decreasing ratios of biomass to solution. The observed increases in specific uptake for lower biomass concentrations (28,29) have been attributed to a shortage of metal concentration in solution rather than interference in binding sites as had originally been suggested (30).

Mass Balance

A mass balance (percentage metal recovery) was calculated as the ratio of metal in equilibrium solution plus the metal retained in the biomass to the measured metal concentration in the initial aqueous solution (Tables 2 and 3). Mean total recovery for all metals at low initial metal concentrations (i.e., 5 mg/L),

Table 2. Mass Balance for Biosorption Experiments with Chitosan at Various Metal Concentrations (1 L Solution and 1 g Biomass)

Metal	Metal Initially in Solution, mg	Metal in Solution After Contact with Biomass, mg	Metal in Biomass, mg	Total Metal Recovery (%)
Zinc	5.7	4.3	0.8	88.9
	10.4	8.7	1.6	93.4
	26.1	21.5	4.5	99.4
	105.0	63.8	52.0	110.3
	194.2	117.0	68.2	95.3
	376.9	268.9	80.6	92.7
Copper	5.4	0.4	3.6	75.4
	10.2	1.1	6.3	70.4
	26.6	2.4	17.8	76.1
	104.1	20.5	61.2	78.4
	206.1	76.5	81.5	76.6
	394.3	256.9	101.7	91.0
Arsenic	5.2	2.8	1.4	82.1
	10.4	7.5	2.5	96.3
	27.3	22.5	4.6	99.5
	108.7	94.3	12.5	98.3
	218.7	185.7	20.7	94.4
	430.3	372.3	33.7	94.4
Chromium	5.3	1.5	2.6	77.6
	10.5	5.3	4.4	92.5
	25.5	16.5	8.8	99.1
	105.0	66.6	28.9	91.0
	206.0	131.7	57.6	91.0
	406.7	282.7	102.8	94.8

All values in the table represent the mean of 3 samples.

Table 3. Mass Balance for Biosorption Experiments with *R oryzae* at Various Metal Concentrations (1 L solution and 1 g biomass)

Metal	Metal Initially in Solution, mg	Metal in Solution After Contact with Biomass, mg	Metal in Biomass, mg	Total Metal Recovery (%)
Zinc	5.8	2.0	2.7	80.4
	11.8	5.0	4.7	82.2
	26.8	17.3	7.7	93.3
	102.6	86.9	11.3	95.7
	199.0	183.6	11.9	98.2
	390.0	376.1	13.7	99.9
Copper	5.0	1.3	2.7	80.9
	10.0	2.7	5.5	81.9
	24.7	11.0	10.1	85.5
	100.0	80.2	16.2	96.4
	194.0	174.8	16.7	98.7
	390.2	366.8	17.0	98.4

All values in the table represent the mean of 3 samples.

where small weight differences would be expressed as large percentage differences, was 80.9% (±4.6%). Mean total recovery from initial metal solutions at 400 mg/L was 95.2% (±3.4%).

Biomass Stabilization

References from the literature (24,31) and the results from our baseline study suggested that metal uptake with anions such as AsO_4^{2-} and $CrOO_4^{2-}$ would be greater if sorption was carried out under low pH. However, the dissolution of chitosan at pH 2 made this impossible to achieve and thus chitosan was stabilized for use at low pH. Initial attempts to stabilize chitosan for use at low pH, by cross-linking with formaldehyde (2% and 37%) following previously described methods (6,9), were unsuccessful. Biomass stabilization at low pH was achieved by cross-linking with benzoquinone under alkaline conditions. However, the sorption capacity for the stabilized chitosan was slightly reduced. Under alkaline conditions, benzoquinone polymerizes but at pH values below 8.0 it forms addition products with amines (32,33) and mercaptans (34). The reduced binding capacity may be due to the reaction of benzoquinone with some of the chitosan amino groups.

When compared under conditions determined as optimum in initial experiments, stabilized chitosan showed greater metal uptake than did *R oryzae* or chitin (Fig. 4). Metal uptake was slightly lower for Cu than that observed in the initial experiments and was lower than predicted for Cr at low pH. For Zn and As, metal uptake was much lower with the stabilized chitosan. The results presented in Fig. 3b were obtained at pH 4 whereas Fig. 4 represents uptake under optimal conditions for each metal as determined from the outcome of initial experimentation. For Cu and Zn the optimum pH for metal uptake was 5, while for As and Cr, the greatest metal uptake occurred at pH 2. Uptake of Cr (125.4 mg/g) is over 5 times greater than the highest values reported for active sludge (24 mg/g) or for free or immobilized *R arrhizus* in a stirred tank or fluidized bed reactor (23–24 mg/g) (35). The removal of Cr observed in our study was 25 times greater than the highest values reported for uptake by a single organism (3–5 mg/g) (19). The increased resistance of the stabilized chitosan to low pH and the high uptake of Cr show the potential for applications in extremely acid conditions. Recent studies with *Sargassum* biomass (23) proposed 2 simultaneous processes: anion exchange for the sorption of Cr(VI), in which the protonated and weakly basic groups in the biomass take up $HCrO_4^-$ ions from solution in exchange for OH^-, and Cr(VI) reduction to Cr(III).

A tenfold increase in UO_2^{2-} loading capacity was reported when *R arrhizus* biomass was compared with pure chitin (36). We observed similar effects with copper and zinc although the removal of copper was approximately 5 times greater with the fungal biomass than it was for chitin, while for zinc the increase was 1.5 times greater with the fungal biomass.

Simulated Mine Effluents

The use of biomass for metal recovery from waste streams for environmental protection requires that biosorption of metal ions be carried out in the presence

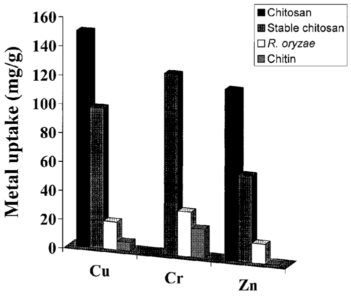

Figure 4. Uptake of Cu, Cr, Zn and As with stabilized (cross-linked) chitosan, *R oryzae*, chitin and chitosan from aqueous solutions (400 mg/L) using biomass concentrations of 1 g/L. Copper and zinc uptake was determined at pH 5 while chromium and arsenic were determined at pH 2.

of multiple ions. The influence of the solution composition and the physicochemical characteristics of targeted metals on biosorption must be considered. Alkaline earth elements have been shown to alter solution ionic equilibrium, with calcium having a negative effect on zinc uptake while magnesium and sodium have shown positive effects on metal uptake (37). Mean concentrations of Ca, Na, Sr, or Mg were not altered after 24-hour contact with chitosan in the simulated copper/zinc or gold mine effluents. These elements were not taken up by the biomass and are probably not involved in ion-exchange reactions between these ions and structural components of the biomass.

Our preliminary work showed the potential for chitosan to remove metals from solution in competitive adsorption systems. Copper, aluminum, and zinc concentrations in the copper/zinc effluent respectively decreased 73%, 14%, and 36%, which corresponded to respective metal uptake values of 16, 11, and 21 mg/g (Fig. 5a) following sorption with chitosan. Aluminum, arsenic, and copper respectively showed 85%, 30%, and 92% decreases in mean concentrations, corresponding to respective metal uptake values of 3.0, 6.0, and 1.6 mg/g (Fig. 5b). Little work has been done on removal of low concentrations of metals from solutions.

Higher removals and higher metal loading on the biomass could potentially be achieved in a multistage system. Existing removal processes such as precipita-

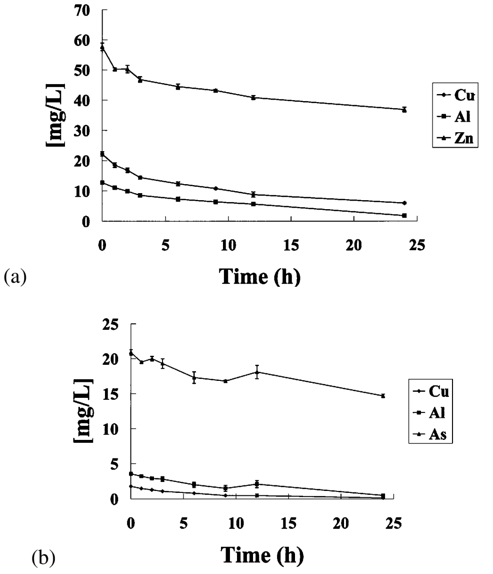

Figure 5. Metal uptake from simulated mine effluents with chitosan (1 g/L) at pH 4. (a) Cu, Al and Zn uptake from a simulated copper/zinc mine effluent. (b) Cu, Al and As uptake from a simulated gold mine effluent.

tion, evaporation, solvent extraction, and membrane processes are most suitable when concentrations are high, becoming either ineffective or expensive at low metal concentrations. The reduction of copper concentrations from 1.77 to 0.14 mg/L is worthy of further study as compliance levels for the tested metals are often below concentrations of 1 mg/L. Bioremediation of abandoned polluted industrial sites often requires the removal of low concentrations of toxic metals from high volumes of wastes. Removal of low metal concentrations may also be applicable in hydrometallurgy for recovery of precious and/or strategic metals.

CONCLUSIONS

The initial pH of the metal solution significantly influenced metal uptake capacity. *R arrhizus* biomass was more resistant than it was with chitosan to the effects of low pH. The increasing metal uptake capacity observed with increasing metal ion concentration for all of the metals examined using chitosan and for copper with the *R oryzae* biomass is a desirable trait for a biosorbent. The optimum biomass loading for metal uptake was 1 g/L. The highest metal uptake values (137, 108, 58, and 124 mg/g respectively for copper, zinc, arsenic, and chromium) were achieved with chitosan. Stabilization of chitosan at low pH was achieved through cross-linking with benzoquinone. Using a commercial source of chitosan, copper concentrations in a simulated effluent were reduced to less than 1 mg/L. Continuing studies are addressing the improvement of biomass quality for polishing applications and recovery of selected metals with subsequent regeneration of biomass.

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