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Accumulation of Cadmium, Lead, and Nickel by Fungal and Wood Biosorbents

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ABSTRACT

Native fungal biomass of fungi *Absidia orchidis, Penicillium chrysogenum, Rhizopus arrhizus, Rhizopus nigricans,* and modified spruce sawdust (*Picea engelmanii*) sequestered metals in the following decreasing preference pb > Cd > Ni. The highest metal uptake was q_{max} = 351 mg Pb/g *A. orchidis* biomass. *P. chrysogenum* biomass could accumulate cadmium best at 56 mg Cd/g. The sorption of nickel was the weakest always at < 5 mg Ni/g. The spruce sawdust was modified by crosslinking, oxidation to acidic oxoforms, and by substitution. The highest metal uptake was observed in phosphorylated sawdust reaching q_{max} = 224 mg Pb/g, 56 mg Cd/g, and 26 mg Ni/g. The latter value is comparable to the value of nickel sorption by wet commercial resin Duolite GT-73. Some improvement in metal uptake was also observed after reinforcement of fungal biomass.

Index Entries: Biosorption; cadmium; lead, nickel; fungal biomass; *Absidia orchidis; Penicillium chrysogenum; Rhizopus arrhizus; Rhizopus nigricans;* sawdust sorption.

INTRODUCTION

Certain types of microbial biomass have been demonstrated to possess capacity for concentrating heavy metals (*1*). Exploratory work in screening for metal-sorbing microbial materials has been in progress for

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some time. Although marine algal biomass showed a very promising sorptive potential, e.g., for cadmium (2) and lead (3), the ocean-based seaweed materials are not readily available in landlocked regions. Indications of good metal sequestering properties by waste mycelia from industrial fermentations (4) should not be neglected for both additional ecological and economical reasons.

Several recent papers described sorption of heavy metals on mycelia of *Aspergillus* and *Mucor* sp. (5,6), *Rhizopus* sp. (7–10), *Penicillum* sp. (11), and other fungal species, e.g., yeasts, *Fusarium* and *Trichoderma* sp. (12–14). The application of biosorption potential in heavy metal pollution abatement has been indicated (15) with a priority target appearing to be the fast-growing electroplating industry (16). In the search for further alternative biomass materials with a metal-sorbent potential, sawdust has also been viewed as a biosorbent (17–19). Other polysaccharidic natural materials have been examined, such as modified cellulose (20,21), bark (22,23), and modified starch (24,25). This work reflects the continuing efforts to find suitable biosorbent materials for Cd, Pb, and Ni with aspects to metal-binding site modification.

MATERIALS AND METHODS

Materials

An industrial sample of dried (28°C) *Penicillium chrysogenum* biomass was obtained by courtesy of Hindustan Antibiotics, Ltd., Pimpri, Pune, India. *Rhizopus arrhizus* was cultivated in the laboratory as described earlier (26). Samples of *Rhizopus nigricans* and *Absidia orchidis* came from industrial processes, nonwashed, and dried at 30°C, kindly supplied by Tianjin Pharmaceutical Co., Tianjin, People's Republic of China. The samples of sawdust were of kiln-dried Canadian linden (*Tilia americana*) and spruce (*Picea engelmanii*).

Chemicals

The origin of the chemicals used was mentioned earlier (2,3). Reagent grade acetic acid, 1-chloroepoxypropane, 2-chloroethanesulfonic acid, sodium salt monohydrate, potassium chloride, sodium periodate, sodium hypochlorite solution, and technical grade sodium chlorite were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Phosphoric acid (anaytical-grade) was obtained from Fisher Scientific (Fair Lawn, NJ).

Methods

Mycelium of *P. chrysogenum* and spruce sawdust were crosslinked with formaldehyde under nonbuffered (27) or pH 2 buffered conditions

(*28*). Final curing was performed at 150°C for 3 min. Mycelium of *R. arrhizus* was crosslinked with 1-chloro-2,3-epoxypropane (*29*) and mycelium *P. chrysogenum* with bis(ethenyl) sulfone (*2*). Additional modifications of crosslinked sawdust included oxidation (*30*) to the oxoform by sodium periodate, and to the carboxyl form by sodium hypochlorite and sodium chlorite. The native biomass was also modified by phosphorylation, sulfoethylation, and carboxymethylation (*31*).

The procedures for calculating the metal uptake, the construction of sorption isotherms, and the description of the Langmuir sorption model were described earlier (*2*). A constant pH for the batch equilibrium sorption experiments was maintained at pH 3.5 by hourly adjustments with 0.1*M* NH_4OH or 0.1–0.5*M* in HNO_3 in the case of lead sorption. Nickel-sorption systems were maintained at the same pH value by adjustments with the same concentrations of either NaOH or HCl, whereas NaOH or $HClO_4$ additions were used for the cadmium-sorption experiments. The final equilibrium concentration of metals in aqueous samples was determined by an atomic absorption spectrometer Perkin-Elmer 3100.

RESULTS

The basic comparison of the tested sorbent materials has to be done at the same equilibrium final concentrations (C_f) of the metal in the residual solution. Metal uptakes at equilibrium residual concentrations of 10 and 200 mg metal/L (q_{10} and q_{200}, respectively) were selected for comparison, representing the choice of "low" and "high" metal concentrations. Both types of the selected metal uptake data for the tested materials, those read from experimental sorption isotherms and data calculated from the Langmuir model, are summarized in Tables 1–3. The tables also show the Langmuir constant *b* related to the "affinity" of the sorbent material for the metal sorbate.

Lead

The comparison of different sorbent materials tested for lead uptake can be made from the summary of results presented in Table 1. Corresponding sorption isotherms depicting performance of individual sorbent materials are plotted in Figs. 1 and 2. The biosorbent uptake of lead by native fungal biomass, as judged by the calculated q_{max} values, decreased in the following order: *A. orchidis* > *R. nigricans* > *P. chrysogenum* > *R. arrhizus*. The highest value of q_{max} = 351 mg Pb/g was observed for *A. orchidis* biomass. Judging by all sorption uptake parameters used in this work, there was a noticeable difference in the sorbent performance between the industrial strain of *R. nigricans* and a laboratory propagated strain of *R. arrhizus*, which turned out to have the lowest sorption capacity for lead.

Table 1
Experimental and Calculated Lead Uptake at pH 3.5 by Different Types of Sorbent Materials

Sorbent type	Experimental		Langmuir parameters[a]				Difference[b]	
	q_{10}, mg/g	q_{200}, mg/g	q_{10}, mg/g	q_{200}, mg/g	q_{max}, mg/g	b, $\times 10^2$	q_{10}, %	q_{200}, %
Fungi								
A. orchidis	14	152	12	147	351	0.36	17	3
R. nigricans	13	105	11	98	166	0.72	18	7
P. chrysogenum[c]	13	74	13	71	93	1.60	0	4
P. chrysogenum-CM[d]	11	63	10	63	89	1.19	10	0
P. chrysogenum	10	50	10	50	63	1.85	0	0
R. arrhizus	8	45	8	42	55	1.59	0	7
Spruce sawdust—phosphorylated	90	187	45	187	224	2.49	100	0
Spruce sawdust—CM[d]	21	130	20	128	179	1.25	5	2
Spruce sawdust—COOH[e]	6	97	5	74	223	0.25	20	31
Spruce sawdust—oxoform[f]	6	75	6	75	178	0.37	0	0
Spruce sawdust—crosslinked[g]	16	81	16	74	91	2.07	0	9
Spruce sawdust	0.2	3	0.2	3	15	0.15	0	0
Linden sawdust	2	8	1	8	12	0.85	100	0

[a] q_{10} and q_{200} metal uptake at the residual concentrations of 10 and 200 mg/L, respectively.
[b] $(q_{EXP} - q_{CAL})\ 100\ /q_{CAL}$.
[c] Crosslinked with bis(ethenyl)sulfone.
[d] Crosslinked with formaldehyde and carboxymethylated.
[e] Oxidized with $Na\ IO_4$ and $NaClO_2$.
[f] Oxidized with $Na\ IO_4$.
[g] Crosslinked with buffered formaldehyde (pH 2).

Table 2
Experimental and Calculated Cadium Uptake at pH 3.5 by Different Types of Sorbent Materials

Sorbent type	Experimental		Langmuir parameters[a]				Difference[b]	
	q_{10}, mg/g	q_{200}, mg/g	q_{10}, mg/g	q_{200}, mg/g	q_{max}, mg/g	b, $\times 10^2$	q_{10}, %	q_{200}, %
Fungi								
P. chrysogenum[c]	8	44	8	43	56	1.58	0	2
R. arrhizus	7	26	7	26	30	3.13	0	0
A. orchidis	4	25	4	24	31	1.64	0	4
P. chrysogenum[h]	2	22	2	22	51	0.37	0	0
R. nigricans[i]	3	18	3	18	26	1.14	0	0
R. nigricans	1	12	1	12	19	0.83	0	0
Spruce sawdust—phosphorylated	23	53	23	52	56	7.17	0	2
Spruce sawdust—CM[d]	6	29	6	28	36	1.96	0	4
Spruce sawdust—COOH[e]	7	23	7	23	26	3.73	0	0
Spruce sawdust—SO_3H[f]	2	13	2	12	18	1.06	0	6
Spruce sawdust—COOH[g]	1	7	1	7	11	0.93	0	0

[a] q_{10} and q_{200} metal uptake at the residual concentrations of 10 and 200 mg/L, respectively.
[b] $(q_{EXP} - q_{CAL})\ 100\ /q_{CAL}$.
[c] Washed with distilled water.
[d] Crosslinked with formaldehyde and carboxymethylated.
[e] Oxidized with $Na\ IO_4$ and $NaClO_2$.
[f] Crosslinked and sulfoethylated.
[g] Oxidized with NaOCl.
[h] Directly from the fermentation process.
[i] Crosslinked with 1-chloro-2,3-epoxypropane.

Table 3
Experimental and Calculated Nickel Uptake at pH 3.5 by Different Types of Sorbent Materials

Sorbent type	Experimental		Langmuir parameters[a]				Difference[b]	
	q_{10}, mg/g	q_{200}, mg/g	q_{10}, mg/g	q_{200}, mg/g	q_{max}, mg/g	b, $\times 10^2$	q_{10}, %	q_{200}, %
Fungi								
A. orchidis	1	4	1	4	5	2.13	0	0
R. nigricans	1	4	1	4	5	1.75	0	0
Urea formaldehyde complex	2	3	1	3	3	3.43	33	0
Spruce sawdust—phosphorylated	19	25	19	25	26	27.24	0	0
Spruce sawdust—CM[d]	2	10	2	10	12	2.47	0	4
Spruce sawdust—COOH[e]	3	12	3	12	13	3.35	0	0
Spruce sawdust—crosslinked[c]	1	3	1	3	4	2.27	0	0
Spruce sawdust—oxoform[f]	1	2	1	2	2	5.42	0	0

[a] q_{10} and q_{200} metal uptake at the residual concentrations of 10 and 200 mg/L, respectively.
[b] $(q_{EXP} - q_{CAL})\ 100\ /q_{CAL}$.
[c] Crosslinked with buffered formaldehyde (pH 2).
[d] Crosslinked with formaldehyde and carboxymethylated.
[e] Oxidized with Na IO_4 and $NaClO_2$.
[f] Oxidized with Na IO_4.

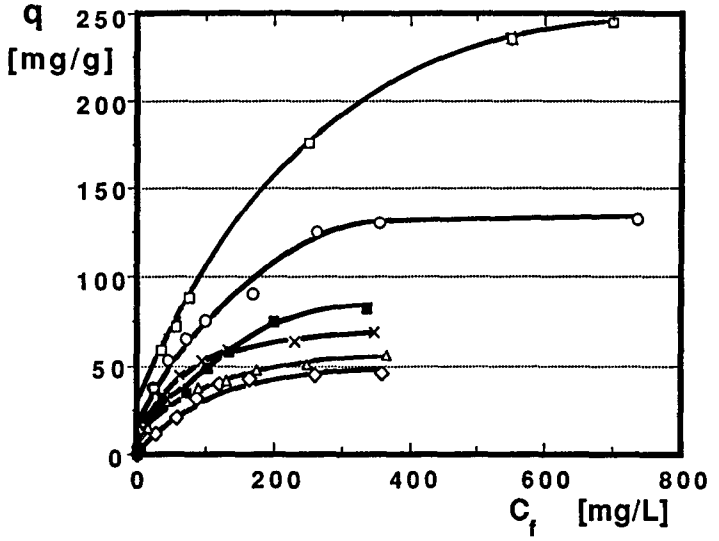


Fig. 1. Lead-sorption isotherms for native and crosslinked fungal biomass (pH 3.5): (○) *R. nigricans*, (□) *A. orchidis*, (X) *P. chrysogenum* (washed), (◇) *R. arrhizus*, (△) *P. chrysogenum* (process raw), (■) *P. chrysogenum* crosslinked with bis(ethenyl)sulfone.

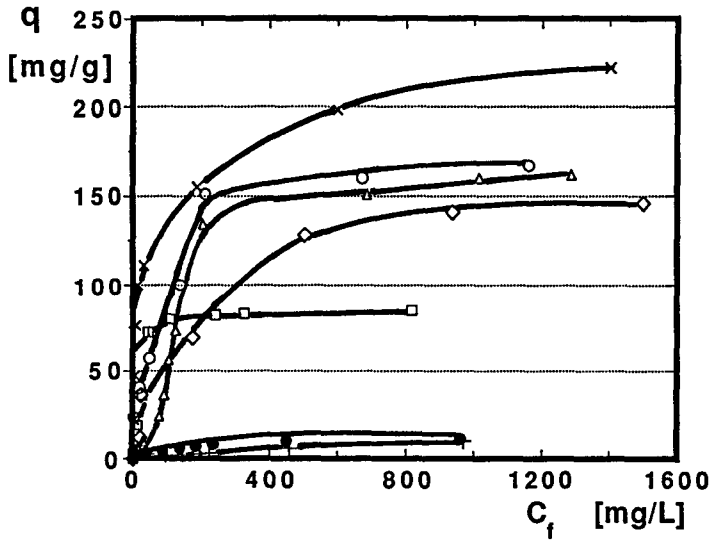


Fig. 2. Lead-sorption isotherms for native and crosslinked sawdust (pH 3.5): (+) spruce sawdust (SSD), (○) SSD formaldehyde crosslinked and carboxymethylated, (□) SSD crosslinked in buffered (pH 2) formaldehyde, (X) SSD phosphorylated, (◇) SSD—oxoform, dialdehyde, (△) SSD—crosslinked with $NaClO_2$ oxidized, (●) linden sawdust.

The maximum lead uptake by native *P. chrysogenum* biomass placed it between the values of both mentioned strains of *Rhizopus*. The attempt to improve its sorption performance by crosslinking with simultaneous introduction of ionic groups was successful. According to the values of q_{max}, the biomass crosslinked by means of bis(ethenyl)sulfone and carboxymethylated material showed an increase lead uptake by 48 and 41%, respectively.

The resulting lead-sorption isotherms followed the Langmuir model reasonably well. When compared to the calculated values, the experimental values of metal uptake q_{10} were 10–18% higher in *A. orchidis*, *R. nigricans*, and carboxymethylated *P. chrysogenum*.

Sawdust was used in a simple test study to indicate the ways of improving the lead-sorbent uptake. The metal uptake by native sawdust of linden as well as of spruce was extremely low; the former "softer" material reached marginally higher values than the latter. The data in Table 1 demonstrate the effect of sawdust modifications on increasing metal uptake capacity in the following order (according to q_{max}): raw sawdust < crosslinked sawdust in buffered formaldehyde < oxoforms after periodate oxidation < carboxylic forms of oxoforms after chlorite oxidation < carboxymethylation after formaldehyde crosslinking < phosphorylation. The corresponding sorption isotherms for the sawdust materials studied are presented in Fig. 2. Phosphorylated sawdust outperformed all other biomass types as judged by any of the uptake criteria (q_{10}, q_{200}, q_{max}, b). It is interesting to note the differences in shapes of some isotherms in the lower C_f range, particularly the conspicuous S-shape isotherm form for the COOH-spruce sawdust material.

Cadmium

When compared to the sorption of lead, the q_{max} values for the uptake of cadmium were almost the same for the native biomass of *P. chrysogenum*, which was the best sorbent for cadmium. The same comparison for *A. orchidis* shows an order of magnitude higher maximum uptakes for lead than for cadmium, whereas those for *R. arrhizus* are 2 to 5.5 times higher. The cadmium uptake by the materials examined is summarized in Table 2 showing the following decreasing q_{max} sequence for native biomass type: *P. chrysogenum* > *A. orchidis* > *R. arrhizus* > *R. nigricans*. Washing of the biomass samples with distilled water may result in an improved metal uptake as indicated in the case of *P. chrysogenum* biomass, which originated from industry. Although crosslinking had little effect on the cadmium sorption, some improvement of cadmium uptake was observed after crosslinking *R. nigricans* biomass with 1-chloro-2,3 epoxypropane. All the resulting key experimental sorption isotherms are plotted in Fig. 3.

Cadmium uptake by sawdust and its derivates is well summarized in Fig. 4. It was also generally up to six times lower in comparison with that

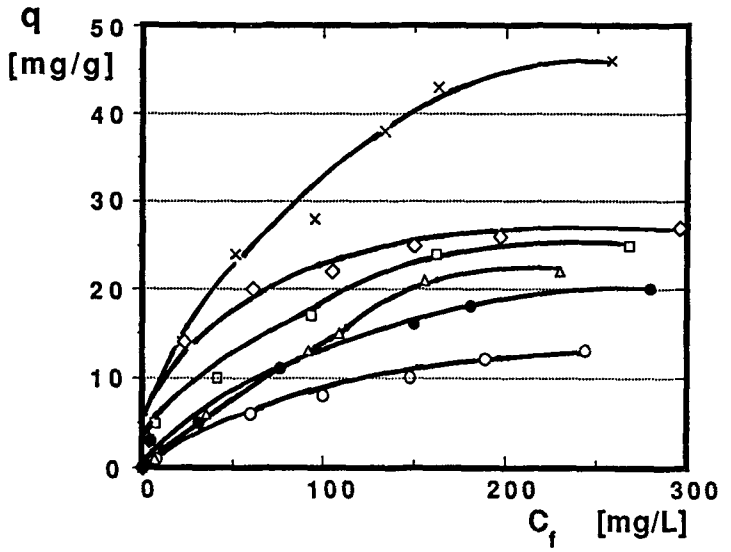


Fig. 3. Cadmium-sorption isotherms for native and crosslinked fungal biomass (pH 3.5): (○) *R. nigricans*, (□) *A. orchidis*, (X) *P. chrysogenum* (washed), (◇) *R. arrhizus*, (△) *P. chrysogenum* (process raw), (●) *R. nigricans* crosslinked with 1-chloro-2,3-epoxypropane.

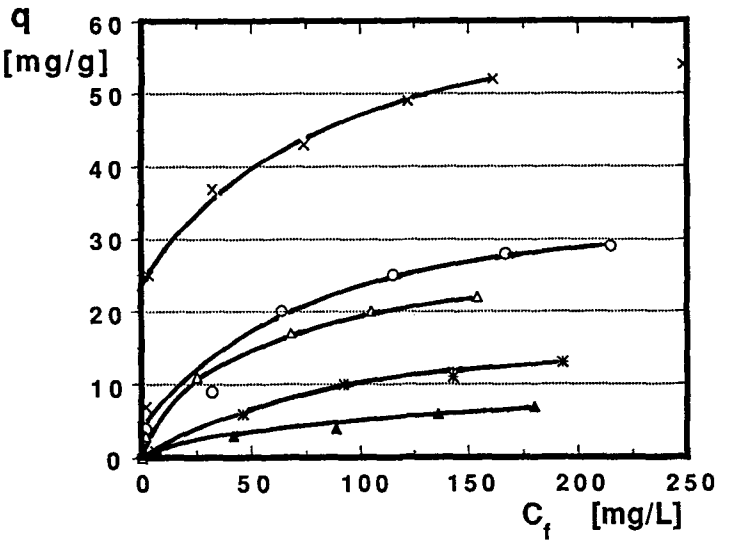


Fig. 4. Cadmium-sorption isotherms at pH 3.5 for spruce sawdust (SSD): (○) SSD formaldehyde crosslinked and carboxymethylated, (▲) SSD—crosslinked with NaOCl oxidized, (X) SSD phosphorylated, (*) SSD—crosslinked and sulfoethylated, (△) SSD—crosslinked and $NaClO_2$ oxidized.

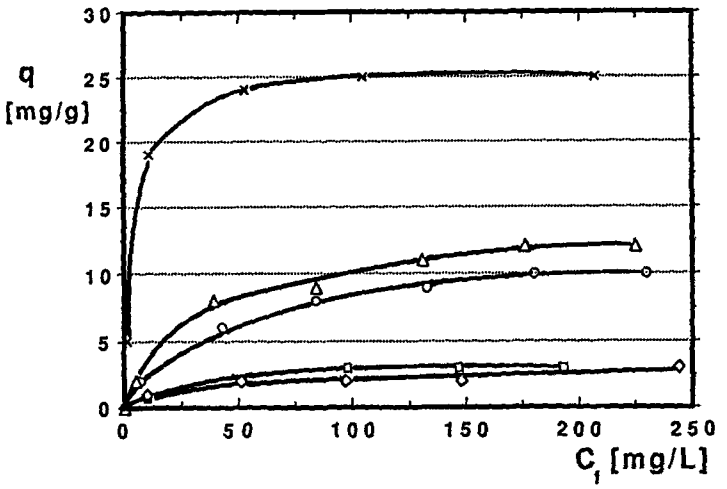


Fig. 5. Nickel-sorption isotherms at pH 3.5 for spruce sawdust (SSD): (○) SSD formaldehyde crosslinked and carboxymethylated, (□) SSD—crosslinked in buffered (pH 2) formaldehyde, (X) SSD phosphorylated, (◇) SSD—oxoform, dialdehyde, (△) SSD—crosslinked and $NaClO_2$ oxidized.

for lead. Cadmium uptake by selected substituted derivatives of spruce sawdust crosslinked by formaldehyde showed the following increasing uptake sequence as judged by q_{max}, in terms of the chemical treatment (Table 2): hypochlorite oxidation < sulfoethylation < chlorite oxidation after periodate oxidation < carboxymethylation < phosphorylation.

Although raw spruce sawdust exhibited negligible cadmium-sorption uptake, phosphorylated spruce sawdust reached higher experimental cadmium uptake values of q_{10} (187%) and q_{200} (20%) than the best fungal biosorbent (washed *P. chrysogenum*). However, these values were still one order of magnitude lower in comparison with the best seaweed biosorbent *Ascophyllum nodosum* (2).

Nickel

Table 3 shows that the sorption uptake of nickel by the materials examined was almost two orders of magnitude lower when compared to their performance with lead. Native mycelia of *A. orchidis* and *R. nigricans* had practically the same extremely low Ni uptake (q_{max} = 5 mg Ni/g biosorbent) that was comparable to the uptake of the urea-formaldehyde composition. However, modified sawdust exhibited one order of magnitude higher uptake than fungal biomass. The increasing uptake followed this sequence based on the chemical modifications (Fig. 5): periodate oxidation of crude sawdust < formaldehyde crosslinking under buffered pH 2 < carboxymethylation < chlorite oxidation < phosphorylation. In summary, the results indicated that in the three metal sorptions examined, the uptake by fungal and sawdust materials always exhibited the following decreasing sequence: Pb > Cd > Ni.

DISCUSSION

The value of q_{max} = 351 mg Pb/g for *A. orchidis* biomass exceeded the q_{max} value of the best native algal biosorbent *A. nodosum* (q_{max} = 272 mg Pb/g) (3) by 29%, but the values of experimental and calculated q_{10}, q_{200}, and *b* were less favorable for *A. orchidis*. The values of q_{10}, q_{200}, q_{max}, and *b* for lead sorption by phosphorylated sawdust exceeded those of many kinds of biomass examined so far and reached the values observed with some brown algal biomass whose sorbent performance is quite comparable with the commercial ion-exchange resin Duolite GT-73 (3).

Cadmium uptake of the examined materials could be labeled as "medium" in general. Nickel uptake was one order of magnitude lower in comparison with that for lead in algal biomass (2). According to q_{max}, the nickel uptake of phosphorylated sawdust was practically identical to that by native algal biomass of *Chondrus crispus* (*Rhodophyta*), *Sargassum fluitans, Sargassum natans,* and *Fucus vesiculosus* (*Phaeophyta*) (3). The experimental and calculated values of nickel uptake q_{10} for phosphorylated sawdust exceeded that of the abovementioned algal strains by one order of magnitude.

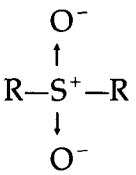
The cadmium and nickel sorption uptake values are generally much lower than those observed for seaweed materials (3) and do not stimulate more interest. Fungal biomass of *A. orchidis* exhibited an excellent uptake of lead. There are several reasons why this fungus, frequently used in transhydroxylation reactions of methylpyridines (32) or steroids (33), could bind heavy metals:

1. The presence of ionic groups capable of sequestering metals. There is glucuronic acid in linear (1-4) linked β-D-glucuronans (34) and phosphates as key constituents of the cell wall (35);
2. The presence of cis-oriented hydroxyl groups in α-D-mannans (36) capable of forming chelate complexes with metals;
3. The presence of other —SH groups of various nonstructural compounds. The biochemistry of steroids usually requires the presence of central acylcarrierprotein (ACP) for the synthesis of fatty acids with two —SH groups and wide participation of sulfur-containing amino acids in transmethylation reactions. The transhydroxylation reactions performed by cytochrome P_{450} involve frequent participation of iron–sulfur protein(s). The formation of strong covalent bonds between lead and —SH groups is well known;
4. *p*-Toluenesulfonic acid is known to be used in the process of microbial hydroxlation of steroids (33). When incorporated, it could enhance the metal uptake; and
5. Chitin and glucan belong to the main structural polysaccharides in fungi. The possibility of metal uptake by these compounds might not only be the result of the acetamido groups of

chitin, but also the result of the entrapment of metals in the inter- and intrafibrillar capillarities in both of these biopolymers.

The species of *Penicillia* do not show such a diversity. Structural polysaccharides consist mainly of glucan and chitin (*37,38*), and glycans containing glucose, galactose, mannose, and sometimes organic acids, e.g., malonic acid. Phosphate groups are also abundantly present as parts of galactans not only in cell walls, but also in exocellular polysaccharides (*39*).

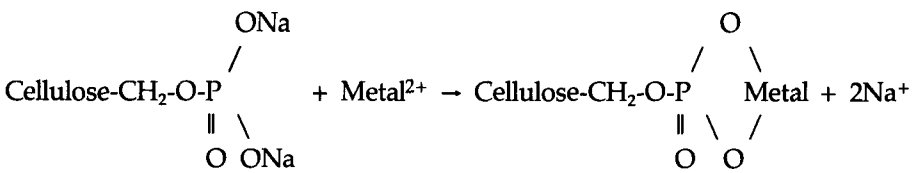
The crosslinking of native biomass with bis(ethenyl) sulfone revealed the possibility of increasing the metal-sorption capacity of native biomaterials by the incorporation of sulfone groups with two coordinate links:



Investigating the case of better sorption of cadmium by *P. chrysogenum* as compared to what was observed here for *A. orchidis* will require further work. According to the structural analysis, the cell-wall D-glucuronans from *A. orchidis* resembled those of *Rhizopus* spp. The differences mentioned above could partially explain the differences in the lead sorption by the two types of fungal biomass, but not the cadmium uptake, which was higher for the *P. chrysogenum* biomass examined in this work.

The present data for metal uptake by *R. arrhizus* are comparable to the ones for lead and cadmium (based on q_{max}) published recently (*9*). However, the industrial strain of *R. nigricans* used in the present work reached the value of lead uptake almost three times higher, and only a little higher for cadmium, than those reported earlier (*9*). Although in the present nickel-sorption experiments *R. nigricans* was superior to *R. arrhizus*, the observed values were still three times lower than those published recently (*9*). The present experiments were performed at a constant pH 3.5, whereas Fourest and Roux (*9*) used a neutral pH for nickel, pH 5 for lead, and the pH value for the cadmium-sorption system was not mentioned. It is quite possible that if identical pH levels were used in both studies, some of the results might be comparable.

Modifications of cellulosic materials tested in this work demonstrated that these materials, e.g., phosphated sawdust, could successfully compete with fungal biomass in sequestering metals. The mechanisms of ion exchange apparently prevails:



This approach was already used, e.g., for thorium recovery from monazite in the past (*40*). the oxidation of sawdust to oxo- and carboxy forms, as well as the introduction of carboxymethyl- and sulfoethyl groups, was only tested in this work for the illustration of biomass transformation into a better metal-sorbent material and for pointing to the eventual possibility of studying different metal-sequestering functional groups and metal uptake mechanisms.

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