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ABSTRACT

The revival of interest in biotechnology has fueled research in many sectors of environmental biotechnology. The present paper describes research utilizing adsorbents prepared from wood-rotting mushrooms growing wild in tropical forests. Nine species of mushrooms were screened using copper(II) as the model adsorbate. While may species showed excellent potential, comparable to biosorbents reported in literature, *Ganodernma lucidum* emerged as the best biosorbent. This biosorbent was further developed for use in a packed-bed bioreactor for treatment of rare earth processing effluents. Electron paramagnetic studies confirmed that adsorption is by chemical binding to the biosorbent.

Key Words. Biosorption; Wood-rotting mushrooms; Rare earth industry; *Ganoderma lucidum*; Electron paramagnetic resonance

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INTRODUCTION

An emerging technology for heavy metal pollution control is the utilization of a microorganism as an adsorbent. Broadly termed "biosorption," which encompasses heavy metal uptake by microorganisms alive, dead, or their derivatives, it has grown from an innovative concept in the early eighties (1, 2) to a full-fledged practical commercial technology (3). At present there are at least three centers commercializing biosorbent products (3).

While the biosorption potential of a number of laboratory-grown species of fungi have been evaluated for their metal uptake potential (4, 5), the potential of naturally available mushrooms, which also belong to the fungal family, is yet to be thoroughly explored. The earliest observation of heavy metal uptake by fungi, however, was reported almost a century ago (6) and utilized a naturally growing corn smut.

The presence of the extensive biodiversity available in tropical forests has been identified as a treasure box for the emerging field of biotechnology (7). Many species of commercial interest for other areas of biotechnology (agriculture, industrial fermentation, pharmaceuticals) were identified in the vast genetic pool of tropical forests (8). It was therefore considered appropriate to conduct an exploratory search for the presence of potential biosorbents in the tropical forests. Because of the vast number of species available, the scope of the search was demonstrative rather than comprehensive.

The study involved the screening of fruiting bodies of macrofungi for their metal uptake capacity for subsequent use as biosorbents. Fungal fruiting bodies (mushrooms) were considered ideal for evaluation as biosorbents for the following reasons.

1. Many species of the phylum fungi have been demonstrated to possess excellent biosorptive potential (2, 9, 10), and mushrooms belong to the same phylum.
2. Mushrooms grow prolifically and are found in most parts of the world (11). Cultivation of mushrooms is also a highly developed industry, the worldwide demand for cultivated mushrooms being of the order of 3.5 million ton with an estimated economic value of $7 billion (12). Collection, preservation, and marketing of naturally growing mushrooms is a well-established activity in many parts of the world (13). Mushrooms can thus be grown in a laboratory or on an industrial scale, should a need arise. The availability of mushrooms, either from natural or from commercial units, hence will not be rate limiting for adoption of the process. Further, nonedible mushrooms have no com-

peting commercial use, and hence they could be procured economically.

3. Mushrooms are macro in size, tough in texture, and have other physical characteristics conducive for their development as adsorbents without the need for immobilization or the deployment of a sophisticated reactor configuration as in the case of microorganisms.

Though there have not been many reported studies on utilization of fungal fruiting bodies as biosorbents, their potential for heavy metal uptake is not totally unknown. Much effort has been spent in the last decade to investigate heavy metal accumulation in higher fungi of the edible variety, mostly because of the possibility of their entry into humans via the terrestrial food chain (14).

Most reported studies on biosorption are on heavy metals (3), and biosorption has become synonymous with the biosorption of heavy metals. There is no information on the application of biosorption for rare earth elements despite the fact that rare earths belong to the category of heavy metals whether taken from the electronic configuration point of view or the toxicity angle (15). However, not much information is available on the adverse environmental impacts of rare earths, and this might be the reason for the apparent lack of studies employing rare earths. This reflects the limited production and application of rare earths rather than their lack of any detrimental effects. For the population exposed to rare earth pollution, its geographical confinement is a burden because there are no standards rare earth pollution and no specified methods for their removal (16). Medical studies, however, are not lacking. Rare earths can degrade the DNA molecule (17) and have been reported to produce tumors at the site of interaction (18). Rare earths also bond with plasma proteins and accumulate in bones (19). Lanthanum levels are consistently high in the blood of cancer patients, although a direct, convincing cause–effect relationship is yet to evolve (20). The rare earth industry appears to be an ideal candidate for trying out the biosorption process. First, the effluent consists of a mixture of elements, each of which is a pollutant and hence need to be removed. Since the biosorbent is versatile, a single adsorption bed can be employed for all the metals. Second, the effluent concentration of the elements is relatively small, and the traditional methods may not be cost effective. Third, the input to the industry is as concentrated metallic solution, and concentrated elements can be directly pumped back into the process stream so no separate recovery unit is necessary. Most importantly, because the rare earth industry is familiar with the ion-exchange process, the technology shock caused by introducing this new method will be minimal, unlike the cases of the metal processing and plating industries.

The present paper reports the results of an investigation where the wood rotting macrofungi *Ganoderma lucidum* was employed for biosorption of rare earth elements.

MATERIALS AND METHODS

The species of wood-rotting mushrooms were collected from the rain forests of Kerala (India). The freshly picked mushrooms were washed clean, sun dried for 2 days, and ground to a practical size of 890 μm (size range >600 μm and <1200 μm). Specimen samples were sent to the Royal Botanical Gardens (UK) for identification.

Chloride salts of mixed rare earths and individual rare earths were used to prepare the adsorbate solution, and the salts used were of triple nine quality (99.9%) procured from Indian Rare Earths Limited (Udyogamandal, India). Since the rare-earth deposits (monazite ore) in India consists of associated thorium, throrium nitrate was used to simulate a process wastewater from the monazite processing industries.

Analysis of rare earths was conducted by the arsanazo(III) method as suggested by Onishi and Sekine (20). The analysis of thorium was also by the arsenazo method as suggested by Marcenko et al. (21). Analysis of other heavy metals was conducted on using an Inductively Coupled Plasma Atomic Emission Spectroscope (Labtam, Australia).

Preliminary adsorption equilibia studies were made using a concentration range of 0.2 to 1 mM adsorbate [copper(II)] solution and an adsorbent dose of 2.5 g/L. The reaction time was 1 hour.

Batch adsorption studies were conducted using individual and mixed rare earths and thorium as adsorbates. Reaction mixture consisted of 100 mL of 1 mM adsorbate solution and 2.5 g/L adsorbent. The pH of the reaction mixture was maintained by acetate buffer at 4.00 ± 0.10. The reaction mixture, kept in 300 mL sampling bottles, was kept on a Rotay shaker and agitated at 30 rpm for 1 hour. At the end of 1 hour the adsorbent was separated by settling and the supernatant was analyzed for the respective adsorbates.

A downflow fixed-bed reactor of 1800 mm length and 50 mm internal diameter was fabricated. This was filled with adsorbent with a packing density of 10 g/L. A simulated effluent from the rare earth processing industry was prepared (mixed rare earths chloride, 250 mg/L; thorium, 0.1 mM; zinc and lead, 0.01 mM; fluoride, 100 mg/L; phosphate, 500 mg/L). This was buffered at pH 5.00 using 0.1 M actetate buffer. The effluent was passed through the column at a rate of 1000 L/h.

Electron paramagnetic resonance analysis was made of the biosorbent before and after adsorption of the rare earth element dysprosium onto it. This was done to differentiate biosorption from ordinary physical adsorp-

tion occurring with activated carbon. The choice of dysprosium was guided by the fact that of all rare earths, it has the maximum number of unpaired electrons, and so the signal strength as well as other details will be maximum for this element.

RESULTS

Equilibrium experiments followed by mathematical formulation and prediction of the ultimate capacity of adsorbents is the accepted practice for comparison of different adsorbents. In the present study, comparison of adsorbents made out of different species was made by using the Langmuir isotherm.

In its simplest form, the Langmuir isotherm is defined as (23)

$$q_e = \frac{Q_{max} b C_e}{1 + bC_e} \tag{1}$$

where Q_{max} is the maximum number of adsorption sites available on the sorbent, q_e is the equilibrium coverage (number of sites occupied) at an aqueous phase concentration of C_e, and b is a constant.

The maximum uptake capacity Q_{max} can be evaluated from Eq. (1) after conducting a series of adsorption experiments. Equilibria experiments were conducted using all nine of the selected adsorbents with initial adsorbate concentrations varying from 0.2 to 2 mM copper(II). The equilibrium distribution curve can be linearized for the evaluation of Q_{max}, the saturation adsorption capacity:

$$\frac{C_e}{q_e} = \frac{C_e}{Q_{max}} + \frac{1}{bQ_{max}}$$

The maximum uptake capacity of different species is presented in Table 1.

TABLE 1
Maximum Metal Uptake Capacity (Q_{max}) of Different Fungal Species

Species	Uptake capacity, Q_{max} (mmol/g)
Trametes lactenia	0.048
Rigidoporus microporus	0.108
Coriolopsis strumosa	0.113
Daedalea tenuis	0.120
Lenzites malaccenis	0.130
Rigidoporus lineatus	0.163
Lentinus strigosus	0.164
Phellinus xeranticus	0.178
Ganoderma lucidum	0.383

It can be seen from the results that all species adsorb metals but there is considerable variation in the extent of metal uptake. *Trametes lactenia* took up only 0.048 mmol/g copper whereas *Ganoderma lucidum* took up 0.383 mmol/g under identical conditions, the other species having uptake values in between.

Uptake of Rare Earths

The results of batch adsorption experiments are presented in Table 2. It is clear from the table that the biosorbent has an ability to accumulate all rare earths up to a maximum of 0.36 mM/g (for La). The minimum uptake was for Ce (0.30 mM/g).

The results of continuous reactor studies are presented in Fig. 1. The reactor was able to bring down the level of the combined concentration of thorium and rare earths to below levels of detectability (0.001 mM). The column is able to achieve substantial reductions for many hours before it reaches saturation.

The EPR spectrum of biosorbent *G. lucidum* after Dy was adsorbed onto it is presented in Fig. 2.

DISCUSSION

As seen from the preliminary results, it has been established that there exists a positive potential for the development of biosorbents from mushrooms in tropical forests. It is relevant to compare the metal uptake potential of the mushroom species studied with the adsorbents reported in the literature. A brief compilation of the metal uptake potential of some widely reported biosorbents is presented in Table 3. Field-scale units have been developed using some of these as biosorbents, indicating their commercial

TABLE 2
Maximum Uptake Capacity (Q_{max}) of Rare Earths and Copper by *Ganoderma lucidum*

Element	Q_{max} (mmol/g)
La	0.36
Pr	0.32
Nd	0.30
Sm	0.33
Eu	0.33
Gd	0.33
Dy	0.30
Cu	0.38

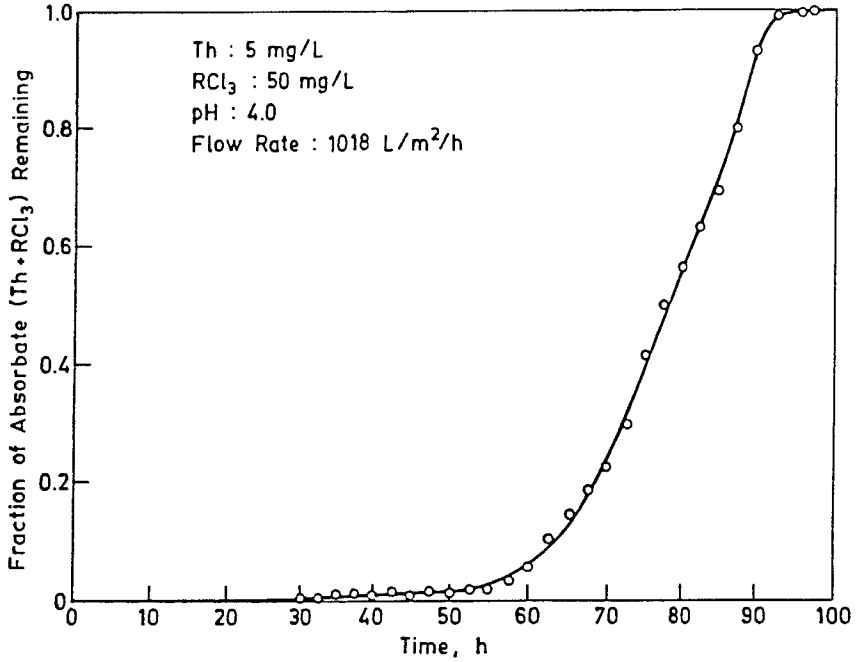

FIG. 1 Breakthrough curve for monazite processing effluent.

viability. *Ganoderma lucidum* exhibited a metal uptake capacity far exceeding all these reported biosorbents. Among nine mushroom species investigated in the present study, eight have exhibited metal uptake capacities greater than 0.1 mmol/g which is comparable to other reported biosorbents. All mushroom species performed better than Filtrasorb 400 (0.030 mmol/g) which is generally employed for heavy metal removal.

The accumulation capacity exhibited by *G. lucidum* for all rare earths is of substantial practical importance. Rare earth effluents invariably contains a mixture of all 14 elements, although in different concentrations. It is therefore essential that any technology intended for pollution control in monazite processing be able to accumulate all of these elements. On that count, *G. lucidum* performs efficiently.

The results for the continuous flow reactor are also of substantial practical significance. Monazite ore always contain traces of thorium in addition to the rare earths. The effluents also contain very high concentrations of phosphates and fluorides. Precipitation, the conventional method of wastewater treatment in the rare earth industry, requires a two-stage process for the precipiation of thorium and rare earths. The presence of an-

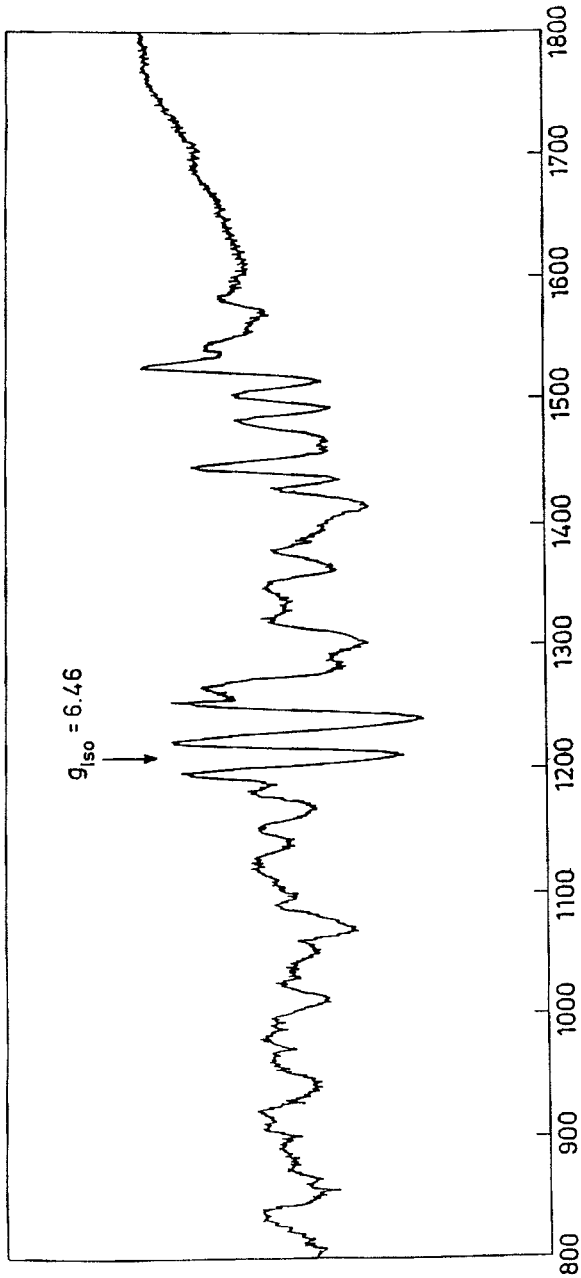

FIG. 2 Typical EPR spectrum of biosorbent after adsorption of the rare earth element dysprosium. Modulation = 10 G, gain = 2×10^4, modulation frequency = 100 K, temperature = 25°C, microwave frequency = 9.21.

TABLE 3
Copper(II) Uptake Capacity of Some Roported Biosorbents

Species	Uptake capacity, Q_{max} (mmol/g)	Reference
Activated sludge	0.125	24
Aspergillus niger	0.120	9
Cladosporium resinae	0.120	25
Penicillium italicum	0.150	25
Penicillium spinulosum	0.040	5
Rhizopus arrhizus	0.250	26
Filtrasorb 400	0.030	Present study

ionic ligands like phosphates and fluorides also affects the precipitation reactor, rendering it virtually impossible to achieve low effluent concentrations. Biosorption by *G. lucidum*, however, is not hampered by any of these difficulties and therefore is an ideal methodology for effluent management in rare earth processing industries.

Rare earths are also paramagnetic species, and hence EPR spectroscopy can be conveniently utilized to verify whether metal uptake is by physisorption or chemical coordination. Although all rare earths except cerium are paramagnetic, the EPR response of only one of the elements is presented as a model. In this context, dysprosium, which has the maximum of five unpaired electrons, was used, and the EPR signal of *G. lucidum* after adsorbing dysprosium is presented in Fig. 2. The EPR spectra of dysprosium is characterized by multiplet signals due to the number of unpaired electrons, its interaction with nuclei, and its interaction with coordinating atoms (26). The EPR of dysprosium is also characterized by an extremely high g value (7.55) for spectra taken in a copper single crystal (27). In the present study the EPR signal is characterized by multiplet signals with a g value of 6.46. The shift in the signal reflected by the change in the g value is indicative of a change in the coordinating environment as compared to the studies reported. The EPR signal clearly indicates that the element is chemically coordinated onto the biosorbent, although no further information regarding the coordinating environment could be obtained.

CONCLUSIONS

The results presented in the this paper supply interesting information regarding the potential applications of wood-rotting fungi for heavy metal pollution control. It is obvious that such a potential exists, but more interesting is the opportunity it affords to investigate the genetic details which

led to this capacity. If such details are identified, the selective transference of such qualities could be tried. The chemical identification of this binding site could be utilized for the preparation of biomimetic adsorbents. The process is being tried at the bench-scale level now and could be commercialized in the future.

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