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Metal Biosorption Studies to Treat Combined Industrial Effluents Using *Phanerochate chrysosporium*

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This work reports the treatability studies conducted on the combined industrial effluent sample by white rot fungus. The selected strain, *Phanerochaete chrysosporium*, a white rot fungus, was employed in reduction of toxic metals. The specific growth rate of the fungus was found to be in the range of 0.089–0.102 hr^{-1}. Studies conducted on biosorption of metals showed that the dead fungal biomass was found to be more effective than living fungus. The optimum pH for the fungal growth was found to be at 4.5 but enhanced biosorption was at pH 6, especially for maximum reduction of hexavalent chromium to trivalent chromium. Laboratory-scale experiments for metal biosorption with this Basidiomycete showed encouraging results, which could be applied further to pilot tests and large-scale studies.

Keywords: Biosorption, mixed industrial effluent, optimum pH, *Phanerochaete chrysosporium,* toxic metal reduction, *and* wastewater treatment.

INTRODUCTION

Biosorption has the following distinct advantages over conventional water treatment methods: does not produce polluting chemical sludge, highly selective, more efficient, easy to operate and, hence, effective for the treatment of larger volumes of wastewater with low metal concentrations.

For metal biosorption studies on a variety of Gram positive and Gram negative bacteria, yeasts, fungi, and algae have been identified, isolated, and screened (Paknikar et al. 1999). Complexation, adsorption, and hydrolysis followed by deposition are the processes that occur during the biosorption of metals (Volesky et al. 1982).

Previous studies had reported on enhanced bisorption by pretreatment with various substances, including boiling water for *S. cinnamoneum* (Puranik 1997), formaldehyde (Galun et al. 1987), Triton X-100 for Penicillium (Palnitkar et al. 1993), and detergent for *S. cerevisiae* (Gadd and White 1989).

Puranik et al. (1995) reported 30.42 mg/g Cd adsorption in the case of 80% ethanol pretreatment for *Streptomyces pimprina*. As sorbent, the repetitive use of wool fibers was found to reduce heavy metal concentrations (Balkose et al. 1992).

Different metal cations and anions are found to be adsorbed by *R. arrhizus* biomass (Tobin et al. 1984). The biomass of *R. arrhizus* at pH 4 exhibited the highest UO_2^{2+} (0.82 mM/g) and Th

biosorptive uptake capacity, in excess of 180 mg/g (Tsezos et al. 1981). At pH 4, the U–*R. arrhizus* biosorption system arrived at a primary equilibrium within the first 60 seconds of contact (Volesky and Tsezos 1982). The removal of Cu^{2+}, Cd^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, and Zn^{2+} from contaminated sources by immobilized biomass of *R. arrhizus* on polyester foam had likewise been reported (Lewis and Kiff 1988).

The above studies have been done for individual industrial effluents, but the application of *Phanerochaete chrysosporium* as a biosorbent for treating combined industrial effluents is unreported. The chosen sample is a representative mixture of effluents from the pharmaceutical, electroplating, paints, and textile industries. The present work attempts to apply *P. chrysosporium*, a well-characterized lignin degrader among white rot fungi, to treat combined effluent samples from several industrial units.

MATERIALS AND METHODS

Chemicals, media, and effluent sample

All chemicals required for the analysis were procured from Qualigens–India, Emerck–India, and SD Fine Chemie–India.

The specified medium for the growth and maintenance of the fungal strain contained Malt extract-20, Glucose-20, Peptone-1, and Agar-Agar-20 g/L of distilled water.

The mineral salt medium employed for the growth and treatability studies contained KH_2PO_4 – 2, $MgSO_4.7H_2O$ – 0.5, $CaCl_2$ – 0.1, NH_4Cl – 0.12, Thiamine – 0.001, and Glucose – 10 g/L of distilled water. The pH of the medium was maintained at 4.5.

Microorganism

The biological system selected for treatment of hazardous compounds present in the effluent sample is a unique Basidiomycete, *Phanerochaete chrysosporium* (MTCC No.787), procured from IMTECH, India.

The culture is maintained on Malt extract agar slants on repeated sub-culturing. The fungal inoculum was prepared by harvesting the spores into saline or distilled water.

Biomass and growth rate determination

The fungal biomass was determined by wet and dry weight analyses. The spores (1 unit of $OD_{650} = 5 \times 10^6$ Conidia/ml) were inoculated into 250-ml Erlenmeyer flasks containing 100 ml of mineral salt medium and incubated at 39°C for 72 h at 150 rpm on a rotary shaker incubator. The flasks were removed at an interval of 8 h and the biomass was collected by filtration foliowed by repeated washings with deionized water and then checked for wet weight and then dry weight by drying in hot air oven at 105°C to achieve a constant weight.

The specific growth rate of the fungus was determined by incubating the culture at 39°C for 72 h at 150 rpm on a rotary shaker incubator. Each 250-ml conical flask containing 100 ml of mineral salt medium, inoculated earlier with 5×10^6 conidia was picked up after every 8 h. The substrate concentration was determined by separating the mycelium from the spent medium by filtration and by estimating the filtrate for reducing sugars by 3,5-Dinitrosalycilic Acid method. The specific growth rate was determined using Monod's equation.

Adaptation and biosorption studies

The effluent sample was a complex mixture of hazardous compounds which formed an extreme heterogeneous environment for the growth of specific strain. A study was conducted to know the growth efficiencies of fungal mycelia and the spores inoculated into undiluted, fivefold-, and tenfold-diluted samples.

A set of flasks containing 100-ml sample was adjusted to pH 4.5 and inoculated with spore suspension and incubated at 39°C/150 rpm. After 5 days and 10 days the flasks were taken out and then filtered. The filtrate was digested with concentrated HNO_3 and the final volume reduced to 20 ml. The sample was filtered and then checked for various metal concentrations by Atomic Absorption Spectrophotometry (AAS Perkin Elmer).

The required biomass was grown in mineral salt medium and harvested by filtration, then washed thrice with deionized water. It was then subjected to drying at 60°C for 10 h and ground

into fine powder using mortar and pestle. The flasks containing tenfold-diluted samples ranging from 30 to 100 ml were inoculated with 0.1 gm of dead biomass. These samples were then checked for metal concentrations by AAS after 90 min of incubation.

In a separate experiment aimed at checking the optimum pH for the conversion of hexavalent chromium to the less toxic trivalent chromium, 0.1 gm of dead fungal biomass powder was added to each flask containing 50 ml of tenfold-diluted sample which had been adjusted to a pH range of 3–6. Then, blanks were run in parallel, or without inoculum. After 90 min of incubation, the samples were filtered and then tested for metal concentrations.

RESULTS AND DISCUSSIONS

The growth profile of the organism is shown in terms of wet weight and dry weight in Figure 1. The fully grown fungal biomass appeared as round puffballs. The increase in biomass was observed along with the increase in incubation period. This was due to the utilization of the primary substrate, glucose, in the defined medium.

Around 97% reduction in weight was observed after drying the biomass in an oven at 105°C for constant dry weight analysis. This rate indicates that the water uptake capacity of the fungus is high; therefore, it can be inferred that the fungus has the capability to sorb most of the pollution load. Maximum fungal growth was observed between 56 and 64 h of incubation in mineral salt medium.

The specific growth rate of the fungus in the mineral salt medium (MSM) was found to be in the range of 0.089–0.102 hr^{-1}, which is of lower value in the effluent sample. The relative decrease in glucose concentration in the MSM with the increase in biomass concentration is shown in Figure 2.

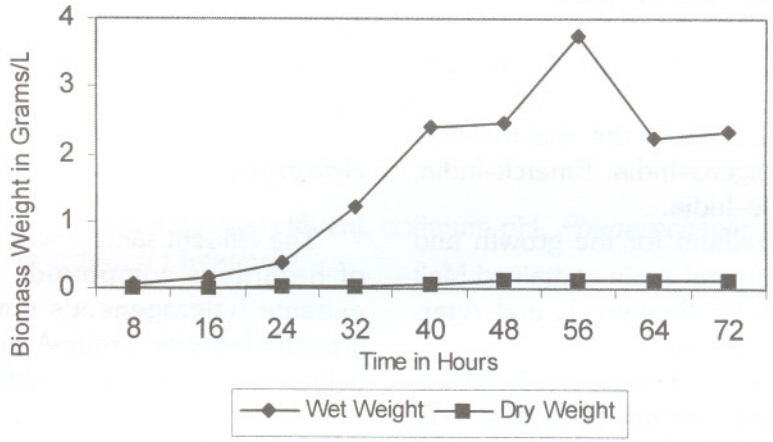

Figure 1. Effect of Biomass Incubation Time on Wet and Dry Weights

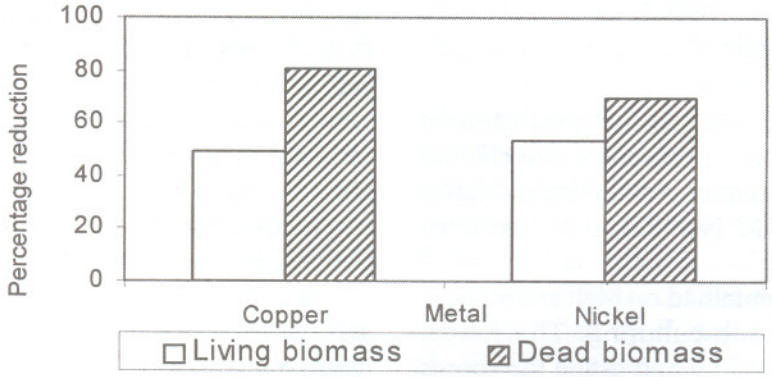

Figure 2. Biomass and Substrate Concentrations vs. Time of Incubation

The lag phase of up to 16 h and the real start of the log phase at 24 h is convenient to measure the rate of reaction. The growth observed between 56 and 64 h, reaching its peak at 64 h, shows that the rapid growth of organisms has taken place at an exponential rate, which indicates the prolific growth rate of the fungus. Though, the specific growth rate value is low in effluent, it appears that the available population in the medium may utilize the pollutants as food and in releasing secondary metabolites.

Optimum temperature and pH for the growth were found to be 39°C and 4.5, respectively. The effluents containing combined metal concentrations in the undiluted and fivefold-diluted effluent samples were high and of inhibitory levels that the environment became unfavorable for fungal growth and metabolism. The growth rate for this sample was compared with those for undiluted, fivefold-, and tenfold-diluted samples. The undiluted and fivefold-diluted samples did not show any sign of fungal growth whereas the tenfold-diluted samples showed the formation of puffballs upon incubation. Figure 3 shows the percentage reduction of metals in the tenfold-diluted samples.

The fungal mycelium was found to be a good matrix for the biosorption of metals. The adsorption capacities of the biomass were found to increase with the increase in incubation time. Table 1 gives the reduction levels in metal concentrations upon incubation with living fungal biomass. The observed variations in the percentage reduction capacity of biomass with respect to the various metals is a function of biosorbent availability.

Figure 4 shows how metal reduction levels are effected by incubation using either live or dead fungal biomass. The percentage reduction levels in copper and nickel were only 49.22 and 53.07, respectively, after 10 days of incubation with living cells; whereas, these values were found to be only 80.42 and 69.30, respectively, after only 90 min of incubation with dead biomass. Dead fungal biomass has exhibited faster and greater biosorption/reduction levels when

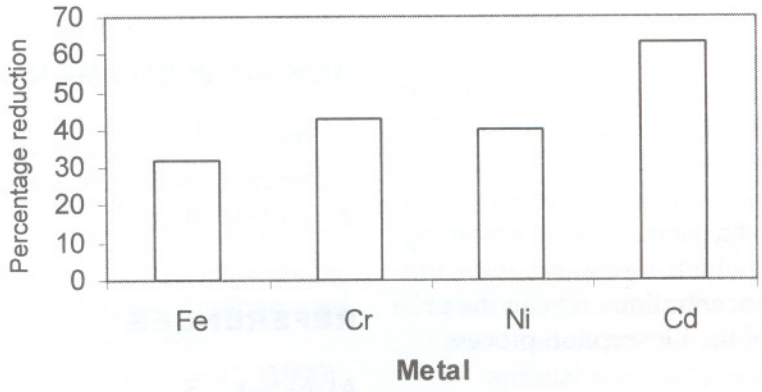

Figure 3. Percentage Reduction in Metals in Tenfold-Diluted Samples

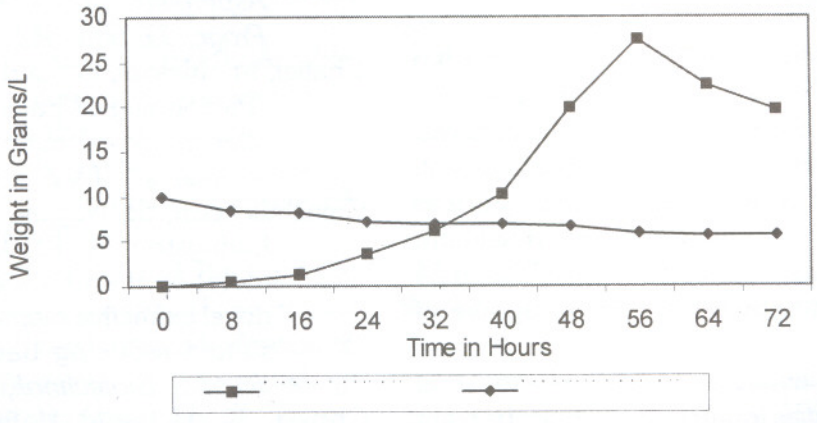

Figure 4. Comparative Metal Reduction Levels with Living and Dead Fungal Biomass

Table 1. Percentage Reduction Levels of Metal Ions with Time of Incubation

Sl No	Metal	Initial Conc. (mg/l)	Percentage reduction after 5 days	Percentage reduction after 10 days
1.	Ni	03.56	23.20	53.07
2.	Mn	20.71	29.52	65.64
3.	Cu	02.67	25.23	49.22
4.	Cd	0.067	52.08	BDL*
5.	Zn	168.6	79.47	85.90
6.	Cr	01.09	24.20	50.42
7.	Fe	70.83	28.66	55.20
8.	Pb	07.00	30.64	39.64

* Below Detection Limits

compared to living biomass at the minimum time of contact.

The probable mild negative charge on the cell surface of the hyphal cells of the fungus adsorbs positively charged metal ions. Here, cell-mediated transformation results in variations in the oxidation state of metal ions. This phenomenon ultimately results in the reduced toxicity of the metals, which can be released into natural streams.

The total chromium present in the effluent samples was checked for reduction levels. The percentage reduction of chromium in the effluent samples was found high at pH 6, as shown in Figure 5. The feasibility of metal ions adsorbing onto the biomass, which decreases with the increase in proton concentrations, reveals the pH-dependant nature of the biosorption process.

CONCLUSIONS

The wood-rotting fungus *P. chrysosporium* has a great potential for application in environmental clean-up technologies. The results obtained reveal the capability of the fungus as a suitable biosorbent.

The specific growth rate of the fungus was in the range of 0.089–0.102 hr^{-1}. Maximum fungal growth was observed between 56 and 64 h of incubation with relative linear uptake of the substrate.

The locally available combined industrial effluent sample was found to show fungal growth when diluted tenfold. When incubated in the effluent sample with various concentrations of different metals, cadmium and zinc reduction was high compared to other metals.

Dead fungal biomass was found to be a more efficient biosorbent than living fungal biomass. The increase in proton concentration in the medium exhibited a degree of reduction in the biosorption efficiency of the fungus.

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