Removal of Cd (II) from Aqueous Solution using Immobilized Rhizomucor Tauricus

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Abstract

An industrial waste fungus Rhizomucor tauricus mycelial biomass was entrapped into alginate gel liquid curing method in the presence of Ca (II) ions. The Biosorption of cadmium (II) by the entrapped live biomass and dead powdered fungal biomass has been studied in a batch system. The binding capacity of immobilized live fungal biomass was very high comparative to dead powdered fungal biomass. The effect of initial metal concentration, pH, temperature and L/S ratio on cadmium removal has been investigated. The maximum experimental biosorption capacities for entrapped live and dead powdered fungal of Rhizomucor tauricus were found to be 79.9 ± 2.2 mg Cd (II) L-1, 57.29 ± 3.4 mg Cd (II) g-1 respectively. The kinetics of cadmium biosorption was slow; approximately 75% of Biosorption takes place in 2 hours. The biosorption equilibrium data were well described by Freundlich adsorption isotherm. The FTIR results revealed that the functional groups -OH and -NH2 involved in the biosorption process.

Keywords: Rhizomucor tauricus; Biosorption; Cadmium; FTIR; Freundlich isotherm

Introduction

The increase in industrial activities has intensified environmental pollution and the deterioration of some ecosystems, with the accumulation of pollutants such as heavy metals, synthetic compounds, waste nuclear liquids, etc. Mining and metallurgical wastewaters are considered to be the major sources of heavy metal contamination, and the need for economic and cost effective methods for the removal of metals.

Many cases of itai-itai disease (osteomalacia with various grades of osteoporosis accompanied by severe renal tubular disease) and low-molecular-weight proteinuria have been reported among people living in contaminated areas in Japan and exposed to cadmium via food and drinking-water. The daily intake of cadmium in the most heavily contaminated areas amounted to 600–2000 μg/day; in other less heavily contaminated areas, daily intakes of 100–390 μg/day have been found (WHO, 1992). The 1963 International Standards recommended a maximum allowable concentration of 0.01 mg/litre, based on health concerns. This value was retained in the 1971 International Standards as a tentative upper concentration limit, based on the lowest concentration that could be conveniently measured. In the first edition of the Guidelines for Drinking-water Quality, published in 1984, a guideline value of 0.005 mg/litre was recommended for cadmium in drinking-water. This value was lowered to 0.003 mg/litre in the 1993 Guidelines, based on the PTWI set by JECFA, (2000). Cadmium accumulates mainly in kidneys and liver, but is also found in skeletal system, muscular system, reproductive system and also in endocrine glands leading to renal dysfunction, hypertension, mutagenesis, and anaemia (Kefala et al., 1999; Kaewsam and Yu, 2001; Park et al., 1999; Nadig, 1990; De Zane, 1990).

Over the years, a number of physical and chemical options such as reverse osmosis, dialysis, gamma-ray irradiation, ultrafiltration, sorbex (adsorption) processes, coagulative (chemical) precipitation, solvent extraction, oxidation (by Ozone and Hydrogen Peroxide), hydrolysis, electrolysis, chromium treatment and chromatography have been contemplated to remediate the effluents (Alguacil and Villegas, 2002; Ritchie and Bhattacharyya, 2002; Verbych et al., 2005; Grimm et al., 1998; Makhlooui et al., 2000; Matlock et al., 2001; Matlock et al., 2002; Esalah et al., 2000; Byrne et al., 1999; Memek et al., 2006; de Martins et al., 2006; Fabiani et al., 1996). But, most of them happen to be tough or hazardous or cumbersome, complex, incomplete or less effective, expensive and commercially unviable moreover requiring lot of energy, reagents, etc. However, these methods are highly expensive when the contaminant concentrations are in the range of 10-100 mg/L (Patterson, 1997). Using microorganisms as adsorbents for removal of heavy metal offers a potential alternative to existing methods. Biosorption, as it has been perceived thus far, could be considered for its economic edges as a possible alternative technique for metal removal/recovery. Biosorption uses biomass raw materials which are either abundant (seaweed) or wastes from other industrial operations (fermentation industry) (Volesky, 1999).

Application of fungal biomass to remove/recover heavy metals from industrial wastewater is economically valuable, technically sound, socially acceptable and is attractive for industry. Many fungal species such as *Mucor melethi* (Guihal et al., 1992), *Aspergillus carbonarius* (Ascheh and Duvanjak, 1995), *Aspergillus niger* (Kapoor et al., 2001; Srivastava and Thakur, 2005;
Park et al., 2005), Rhizopus arrhizus (Prakasham et al., 1999; Sag et al., 2001), Mucor rouxii (Yan and Viararaghan, 2000), Rhizopus nigricans (Bai and Abraham, 2001), Phanerochaete chrysosporium (Say et al., 2001), Trametes versicolor (Bayramoglu et al., 2003; Yalcinkaya et al., 2002), Saccharomyces cerevisiae (Lin et al., 2005), Mucor hiemalis (Ebner et al., 2002; Tewari et al., 2005), Botrytis cinerea (Akar and Tunali, 2005), Neurospora crassa (Tunali et al., 2005) and Lentinus sajor-caju (Bayramoglu et al., 2005; Bayramoglu et al., 2002) have been extensively studied for heavy metal biosorption and the process mechanisms seems to be different from species to species.

Compared with the free biomass, immobilized microorganisms are considered more suitable for industrial application due to better mechanical strength, chemical stability, physical morphology, anti degradation ability and more importantly, repeated adsorption–desorption characteristics (Tsezos et al., 1989; Lloyd et al., 1997). Natural polymers such as alginate, chitosan, chitin and cellulose derivatives have been mostly used as the matrix for the immobilization of microbial cells via an entrapment technique. A major advantage of alginate gel entrapment is that immobilized cells do not suffer extreme physical–chemical condition changes during the immobilization process. Permeability, null toxicity and transparency of formed matrix imply a very gentle environment for immobilized cells (Smidsrød and Skjåk-Bræk, 1990; Araújo and Santana, 1996). Immobilized fungal cells were found to be far more stable during experimentation in the batch system than the free fungal cells.

Several researchers reported immobilized fungal biomass for biosorption of heavy metal ions using calcium alginate such as Lentinus sajor-caju (Bayramoglu et al., 2002), Trametes versicolor (Bayramoglu et al., 2003), Rhizopus arrhizus (Liao et al., 2004), algal cells C. vulgaris (Al-Rub et al., 2004), Tetraselmis chui (Moreno-Garrido et al., 2005). Some of the workers used dead fungal biomass, eliminates the need of nutrient requirement and can be used as biosorbents, such as Rhizopus nigricans (Bai and Abraham, 2001), Botrytis cinerea (Akar and Tunali, 2005), Mucor hiemalis (Tewari et al., 2005), S. cerevisiae (Lin et al., 2005).

Biosorption refers to different modes of non-active metal active by microbial biomass, where metal sequestration by cells can take place through adsorption, ion-exchange, coordination, complexation etc. This technology is based on the deployment of dead biomass where metal ions binding to various functional groups of the cell wall takes place by passive mechanism (Vegilo and Beolchini, 1997). The -COOH, -NH2, -OH and –SH groups on microbial cell wall provide the binding sites for interaction of metal ions (Kuyucak and Volesky, 1998).

Rhizomucor tauricus is chosen as biosorbent material because it is waste industrial fungus, relatively lack of information about the sorption ability. In this investigation live immobilized and dead powdered fungal biomass of R. tauricus was used for the removal of cadmium in aqueous solution. Our studies was aimed at investigating the mechanism of metal uptake of Cd (II) ions from aqueous solution and the role involved by the functional groups present in the biomass in biosorption process was examined by FTIR analysis in addition to the environmental parameters affecting the biosorption process such as pH, time and initial metal concentration, biomass dosage (L/S ratio) and temperature. Comparison between sorption on living and dead free fungal biomass was also investigated. Equilibrium modeling was carried out using Freundlich type of isotherm equation.

Methods

Immobilization of rhizomucor tauricus

The fungus Rhizomucor tauricus MTCC 1976 was purchased from MTCC, Chandigarh. R. tauricus was produced by standard culture technique using Potato Dextrose Broth, incubated for 72 hours at temperature 30°C. The biomass was filtered through vacuum filtration unit to separate the unused nutrient broth. Rinsed twice with distilled water, filtered through vacuum filtration unit and resuspended in distilled water. Sodium alginate (4%) solution was prepared in hot distilled water at room temperature, Predetermined weighed biomass (Wet weight) in alginate solution were mixed thoroughly with magnetic stirrer. The uniform mixture of fungus and sodium alginate solution (2%) was pumped through the peristaltic pump into the 0.5 M CaCl2, 2H2O solution. The beads were stored at 4°C for overnight for cured with 0.25 M CaCl2, 2H2O solution, beads were (4 mm) washed twice with distilled water to avoid the excess CaCl2,2H2O, these beads were used for equilibrium studies.

pH adjustment of aqueous metal solutions

The adsorption of metals decrease at low pH values because of competition for binding sites between cations and protons, while at pH higher than 7, hydroxo species of the metals can be formed and do not bind to the adsorption sites on the surface of the adsorbent. So, pH was adjusted with the range of 3-7 by adding the 0.1N HCl and 0.1N NaOH for acidic and basic pH respectively.

Biosorption studies

Cadmium test solutions of various concentrations of 100 ml each were pipetted out into 250 ml conical flasks. To each of these flasks, 0.1 gram of biomass (dead biomass) was added and shaken in an orbital shaker for a predetermined time in order to attain equilibrium. The flasks were allowed to settle for half an hour and equilibrium pH of the solution was measured. Samples were collected from each flask and analyzed. The procedure was repeated for 0.25g and 0.5g biomass, for different pH, at different temperatures (10°C, 30°C and 50°C). For the immobilized fungus R. tauricus the procedure was repeated for 10 ml volume of beads at the same above conditions. The volume of immobilized biomass was taken by replacement method at known wet weight of biomass.

Analytical procedure

The concentrations of unadsorbed Cadmium ions in the sample supernatant liquid were determined using an atomic absorption spectrophotometer (Perkin Elmer model AA200) with an air-acetylene flame. Cadmium hollow cathode lamp was used and metal uptake (q) was calculated using the general definition:

$$ Q \text{ (mg or m mol g}^{-1}\text{)} = V (C_f - C_i)/M $$

Where C_i and C_f are the initial and final (equilibrium) metal concentrations in the solution respectively, V is the solution volume and M is the mass of the biosorbent used.
FTIR analysis

The powdered biomass before adsorption and after adsorption air dried and the moisture was removed completely at 60°C by humidity control oven. The powder was analyzed by Fourier Transform Infra red spectrophotometer. FTIR studies were conducted at Sipra labs, Hyderabad by potassium bromide (KBr) pellet method in the wave number range of 400.00 Cm⁻¹ to 4000.00 Cm⁻¹. The FTIR is of Perkin-Elmer make serial No. 72425 (Spectrum one FTIR spectrophotometer) to obtain information on the nature of the possible cell-metal interaction.

Results and Discussions

Effect of time

Experiments were conducted to estimate the time required to reach the equilibrium by taking an initial charge of 100 ml of aqueous solution containing cadmium ions and biomass immobilized R. tauricus and thoroughly shaking the mixture using orbital shaking machine. The samples were drawn at different intervals of time and the metal concentration was estimated using Atomic Absorption Spectrophotometer (AAS). The time required to reach equilibrium was found to 3 (Figure 1) and 2½ (Figure 2) hours for dead powdered fungus and immobilized fungus respectively.

Effect of pH

The percent adsorption increased with increase in pH, at near neutral pH 6 of the (Figure 3, Figure 4) solution were found to favor adsorption very strongly. At lower pH the percentage adsorption was very low; it may be due to preferential adsorption of hydrogen ions. These observations were in agreement with those reported in earlier investigations (Senthilkumar et al., 2006; Bayramoglu et al., 2006; Marques et al., 1999; Say et al., 2001; Yan and Viraraghavan, 2003; Vasudevan et al., 2003; Zafar et al., 2007; Bishnoi et al., 2007; Sari and Tuzen, 2008; Murthy et al., 2007; Fagundes-Klen et al., 2007) for different metals using various biosorption materials.

Effect of temperature

The effect of temperature on percent adsorption of cadmium using powdered R. tauricus and immobilized R. tauricus.

It can be observed (Figure 5) that temperature was found to favor biosorption (Kumar et al., 2006). This may probably due to increase in activity of biomass as the temperature increases. The same trend was observed in case of dead fungal biomass.

Effect of different variables on equilibrium distribution

The increase in aqueous metal concentration increased the metal uptake, further the increase in pH also increased the metal uptake. The gradual fall in the metal uptake as the pH is decreased is due to the preferential adsorption of hydrogen ions to metal ions (Figure 6, Figure 7).

At low pH (pH = 3) the equilibrium concentration of adsorbed species was found to be very low as the sites are occupied more by hydrogen ions. Further, with an increase in pH, there is an increasing legends with negative charges resulting increased in binding of cations. These observations ensured the applicability

![Figure 1](image1.png)  
**Figure 1:** Variation of aqueous metal (Cd⁺⁺) concentration with time on dead R. tauricus.

![Figure 2](image2.png)  
**Figure 2:** Variation of aqueous metal (Cd⁺⁺) concentration with time on immobilized R. tauricus.
Correlation of results

The observations from the earlier sections reveal that the biosorption of metal ions essentially depends on surface heterogeneity, surface imperfections, type of biomass material and the functional groups associated with it. Metal adsorption on the solid phase expressed as $C_s$ can be assumed to be a function of metal ion concentration and concentration of other relevant ions present in the aqueous phase, amount of biomass wt./vol., temperature and pH as (3.1):

$$C_s = f(C_A, \text{pH}, T, (L/S))$$ (3.1)

Earlier investigators had used the adsorption isotherm model of Freundlich (Sag et al., 2001; Sari and Tuzen, 2008; Volesky and Holan, 1995; Aksu, 2001; Akar and Tunali, 2005; Preetha and Viruthagiri, 2007; Climino, 2000; McKay and Porter, 1997; Inbaraj and Sulochana, 2004; Altin et al., 1983; Taty-Costodes et al., 2003) and applied them to their data successfully. The Freundlich model is the most commonly employed adsorption isotherm equations to represent the experimental data as they represented their equilibrium data over a wide range of experimental parameters. In view of this an attempt has been made to present the experimental data of present study through an empirical approach, using following Freundlich type of equation. (3.2)

$$C_s = K C_A^n$$ (3.2)

Where $C_s$ is the concentration of metal ion in solid adsorbent expressed as mg/gm of solid and $C_A$ is the concentration of metal ion in aqueous phase expressed as ppm (mg/lit of solution), $K$ and $n$ are constants.

The Equilibrium distribution data was plotted as variation of log $C_s$ with log $C_A$ and shown plotted in Figure 8, Figure 9 $Cd^{2+}$ for biomass materials used. It was found that the equilibrium data were well fitted to a straight line indicating the applicability of Freundlich equation for the entire range of experimental variables of present study.

The intercepts were found to vary with pH, liquid solid ratio and temperature. Therefore the entire data were subjected to regression analysis using FORTRAN programming. The format of equation used for regression is:

$$C_s = a_0 C_A^{a_1} C_H^{a_2} (L/S)^{a_3} 10^{a_4/T}$$ (3.3)

Or

$$\log C_s = \log a_0 + a_1 \log C_A + a_2 \log C_H + a_3 \log (L/S) + a_4/T$$ (3.4)

Where $C_s$ is the dependent variable, $C_A$, $C_H$, $(L/S)$ and $T$ are...
independent variables, \( a_0, a_1, a_2, a_3 \) and \( a_4 \) are constants. The data were well fitted to the above equation by regression analysis for immobilized fungus and dead powdered fungus (3.5 & 3.6).

\[
C_S = 0.5214 C_A^{1.209} C_H^{-0.07807} 10^{(215.3/T)} \tag{3.5}
\]

\[
C_S = 0.001248 C_A^{1.078} C_H^{-0.05859} 10^{(79.69/T)} (L/S)^{0.8889} \tag{3.6}
\]

The regression analysis of the data values for immobilized fungus were 16.71, 21.71 average and standard deviations respectively. Where as in case of dead powdered fungus, the regression analysis of the data values were 18.48, 23.67 average and standard deviations respectively.

**FTIR spectroscopic analysis**

The FTIR spectroscopic analysis of the biomass, dead powder of *R. tauricus* before adsorption of heavy metal ions (Figure 10) indicated broad adsorption band at 3423.28 \( \text{Cm}^{-1} \), representing –OH and -NH stretching, 2925.21 \( \text{Cm}^{-1} \) and 2854.16 \( \text{Cm}^{-1} \) represented –CH stretching. The absorption band at 1742.68 \( \text{Cm}^{-1} \) could be attributed to C=O group of carboxylic acid and absorption band at 1417.14 \( \text{Cm}^{-1} \) representing carboxylate group. Further at 1180.53 \( \text{Cm}^{-1} \) indicating –OH group of sugars and 1076.53 \( \text{Cm}^{-1} \) and 1031.88 \( \text{Cm}^{-1} \) are representing amide C-N stretching and –P=O stretching respectively.

In all the FTIR spectroscopic analysis of the biomass, dead powder of *R. tauricus* after adsorption of heavy metal ions (Figure 11) revealed that the shift of 32 to 40 for the peaks at 3423.23 \( \text{Cm}^{-1} \), 1742.68 \( \text{Cm}^{-1} \) and 1180.53 \( \text{Cm}^{-1} \). It clearly indicated that the carboxylic acid and hydroxylic groups are the main functional groups for complexation of metal ions.

**Conclusions**

Experimental investigations have been carried out to determine the equilibrium distribution of metal ions between aqueous solution containing different metal ions and the fungal (Immobilized and dead powdered) *Rhizomucor tauricus* biomass. The equilibrium adsorption data were obtained varying the concentrations of Hydrogen ion (pH) and other metal ions in solution and also varying the liquid solid ratio and temperature. Based
on experimental data points, the following conclusions were drawn:

1. The time required to reach equilibrium was found to be 2.5 hours for Immobilized \textit{R. tauricus} and 3 hours for dead powdered \textit{R. tauricus}. Increase in aqueous metal concentration increased metal adsorption for all conditions of study.

2. Decreases in pH of aqueous phase decreased metal adsorption due to the preferential adsorption of H\(^+\) ion compared to metal ions. Neutral pH conditions were found to favor metal adsorption.

3. Increasing temperature increases metal adsorption in all cases of study.

4. Industrial fungus \textit{R. tauricus} after adsorption of heavy metal ion cadmium concentration occurred due to the interaction of –OH and –NH.

Since binding capacities are relatively high for both immobilized live and dead powdered fungal forms, those fungal forms could be considered as suitable biosorbents for the removal of cadmium in wastewater treatment.

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