Journal of Civil Engineering
The Institution of Engineers, Bangladesh
Vol. CE 27, No. 2, 1999

BIOSEPARATION OF LEAD (II) IONS FROM WASTEWATER BY USING A FUNGUS P. OSTREATUS

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ABSTRACT : Bioseparation of heavy metals by dead microbial cells has been recognized as a promising alternative to existing technologies for removing heavy metals from industrial effluents. In the present study, the biosorption of lead(II) ions was investigated in a single-staged batch reactor and its practical applicability was studied through a down flow column using lead spiked distilled water. The fruiting bodies of a fungi *Pleurotus ostreatus*, was used as the biosorbent. The batch sorption kinetics can be described by a first order reversible reaction and equilibria follow the Freundlich, adsorption isotherm. The uptake of lead is a function of pH of the wastewater and the pH adsorption edge was found from pH 2 to 4. The increase of ionic strength in solution (using $NaNO_3$) beyond 0.1M, substantially decreases the sorption of lead. The influence of soluble complexing agents such as ethylene diamine tetra acetic acid (EDTA) and other metal cations like calcium and magnesium on the adsorption efficiency of lead by the biosorbent was studied. From the results of the down flow column study, the biosorbent P. ostreatus was found effective in continuous flow system for the separation of lead from both lead spiked distilled water and tap water. Used biosorbent can be regenerated with diluted acid.

KEYWORDS : Biosorption, Pleurotus ostreatus, Adsorption isotherm, Heavy metals, Biosorbent, Mechanism.

INTRODUCTION

Lead is used as an industrial raw material for storage battery manufacture, printing, pigments, fuels, photographic materials and explosives manufacturing. Lead is also a common constituent of plating wastes although is not as frequently encountered as copper, zinc, cadmium and chromium. Lead pollution resulting from mining and smelting has also been reported in the literature. Microbial biomass has a high affinity for heavy metals, as observed both in laboratory

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studies and in natural environments (Gadd, 1986a,b). The sequestration of metals by microorganisms is a significant component of biogeochemical cycling and is also relevant to the fate of potentially toxic heavy metals when discharged into the environment. The ability of microorganisms to accumulate such elements can result in their immobilization and removal from solution but ingestion from other organisms can result in transfer along food chains, ultimately to humans (Gadd and Griffiths, 1978). These properties of microorganisms have given rise to a considerable interest in the use of microbial biomass and derived products to remove metals from industrial wastes (Gadd, 1988, Macaskie, and Dean 1989). Biosorption (where microbes are engaged for the adsorption of heavy metal ions) processes have been proposed as an efficient and potentially cost effective way of removing toxic heavy metals from industrial effluents with metal concentrations in the range of 1-100 mg/L (Volesky, 1990). Fungi may be better suited for this purpose than other microbial groups because of their great tolerance towards metals and other adverse external conditions such as low pH, their high wall binding capacity and their high intracellular metal uptake values. The form of the biosorption is an important consideration when microbial material is used in a metal removal process. For real or simulated industrial application, freely suspended fungal biomass has several disadvantages which include low density and mechanical strength which may make biomass/effluent separation difficult (Tsezos, 1986). The unicellular form of many microbes, such as bacteria, yeasts or microalgae, presents a problem in any reactor system where the treated process stream flows continuously through the reactor, as they may be subject to washout, compaction or clogging (Gadd, 1986 and Tsezos, 1986). Dead fungal cells can bind metals at levels greater than, equivalent to or less than live cells depending on the method used to kill the cells (Sakaguchi et. al., 1979). The mechanism of biosorption of metals can vary considerably between species and on whether organisms are living or dead and also depend on the nature of metals. Gadd and Griffiths (1978) identified two mechanisms which enables microorganisms to concentrate metals: binding of metals to cell surface components and intracellular accumulation. Surface and wall binding is rapid, often taking less than a few minutes and the amount bound can be large. Biosorption capacity of fungus V. volvacea for cadmium uptake was found to be varying from 9.13 to 9.33 mg/g for different sizes of sorbent (Osman and Bandyopadhyay, 1996). Some researchers investigated the removal characteristics of four fungal species both growing and dead and found that Phanerochaete chrysosporium were among the most effective for Pb(II) removal (Dey et. al., 1995). Both adsorption and precipitation of hydrolysis product occur with uranium (Tsezos and Volesky, 1982a), while coordination with cell wall nitrogen was the main mechanism of thorium biosorption (Tsezos and Volesky, 1982a, b). The purpose of this research work was to study the lead uptake properties of locally growing fruiting bodies (mushroom) of fungus Pleurotus ostreatus and the influence of different factors on lead removal and also to desorb the adsorbed lead ions from the biosorbent for the possible reuse and or for safe disposal.

MATERIALS AND METHODS

Biosorbents and Chemical Reagents

Locally cultivated mushroom grow prolifically in humid temperate climate and maximum growth was observed during the post monsoon period. The mushroom were therefore collected during the month of October to December from Rural Development Centre (RDC), IIT Kharagpur. The fruiting bodies were harvested from the culturing bed made of paddy straw, washed in water and sun dried for two days before biosorbent preparation. After drying, the fruiting bodies were pulverized to different particle sizes and were collected for biosorption studies. A geometric mean size of 0.595 mm was used in all experiments except for isotherm studies. Metallic salts used in this work were nitrates of lead, cadmium, calcium and magnesium, and metal powders were used for copper and zinc. All metallic salts and chemicals were of analytical grade. Metal estimation was carried out by using AA-670, Atomic Absorption/Flame Emission Spectrophotometer (Shimadzu, Japan).

Biosorption Studies in Batch Reactor

Batch uptake experiments of lead by untreated biosorbent were conducted at room temperature in gyratory shaker for an effective solid-liquid contact using 150 mL plastic bottle. Kinetic studies on the interactions between Pb (II) and P. ostreatus were performed under completely mixed conditions. Samples were separated from the shaker at predetermined time intervals and supernatants were collected by gravity settling and analysed for residual Pb(II) concentrations. The sorption isotherm was conducted using different particle sizes of biosorbent with different initial lead concentrations. The effect of pH of the suspending medium on sorption of lead was studied by adjusting the solution pH with 0.1N HCl or 0.1N NaOH in the range of pH 2.0 to pH 6.0. The effect of ionic strength was studied using $NaNO_3$ in the range of 10^{-4} M to 1.0 M. The effect of EDTA on lead removal was studied by varying EDTA concentration from 0 to 0.001M in a 10 mg/L initial lead solution. The effect of calcium and magnesium ions on lead removal was studied in the range of 0 to 2.5 mM for calcium and 0 to 2 mM for magnesium. Nitrate was used because of its poor ability to complex metallic cations (Msaky and Calvet, 1990).

Desorption Study

A 800 mL reaction mixture containing 2 g/L biosorbent was agitated in a 1 L beaker in the presence of 10 mg/L lead. After 3 hours contact time the supernatant was analysed for lead. The biosorbent was then separated and washed with distilled water and dried. A desorption study was carried out by taking 0.5 gm of Lead (II) saturated biosorbent in (1) 50 mL of distilled water (2) 50 mL of 0.01N hydrochloric acid and (3) 50 mL of 0.1N hydrochloric acid. The supernatants were analysed for lead after 3 hours of contact time.

Column Study

Column studies were done using lead spiked tap water and distilled water. The characteristics of tap water is shown in Table 1. A 1.4 cm I.D. glass column was filled with biosorbent (2.51 gm geometric mean size of 0.596 mm) to a height of 10.25 cm. Glass wool was used at the bottom of the column to support the sorbents. A constant flow rate of 10 mL/min ($3.9\ m^3/m^2$-h) was maintained. The above test conditions were maintained for both tap water and distilled water. The samples from the influent and effluent were collected periodically and analysed for lead.

Table 1. Characteristics of Tap Water.

Parameters	Values
pH	7.1 - 7.3
Turbidity	5 NTU
Conductivity	2.48×10^{-3} mho/cm
Hardness (Total)	83 mg/L as $CaCO_3$
Calcium	23 mg/L as Ca^{2+}
Magnesium	4 mg/L as Mg^{2+}
Sodium	5 mg/L as Na^+
Chloride	23 mg/L as Cl^-

RESULTS AND DISCUSSION

Biosorption kinetics

Metal uptake often follows a two-step kinetics. Same phenomenon was observed when the data from kinetic studies were plotted in Fig.1. It was seen that the biosorbent take up 62% (3 mg/g) of the total amount of Pb(II) (4.9 mg/g) within the initial five minutes. Pb(II) uptake continues even till the end of 360 min. at a very slow rate. This implies that the extracellular association (surface binding) occurs first and takes place rather fast. Tsezos and Volesky (1982a,b) have found that uptake of Uranium and Thorium by dead R. arrhizus reaches a plateau within 1 min. They have also suggested that surface binding is the key to the rapid establishment of an uptake equilibrium. The sorption of lead from liquid phase to solid phase may be considered as a reversible reaction with an equilibrium being established between two phases. A simple reversible first order kinetic model was, therefore, used to establish the rates of reaction of adsorption of an adsorbate from solution (A) to sorbent (B), which can be expressed as

$$A \underset{K_2}{\overset{K_1}{\leftrightarrows}} B \qquad (1)$$

Where k^1 and k^2 represent the forward and reverse rate constants. If X represents the amount of lead conversion, then :

$$-dC_A/dt = dC_B/dt = dx/dt = k_1 C_A - k_2 C_B$$

$$= k_1 (C_{A,0} - X) - k_2 (C_{B,0} + X) \qquad (2)$$

$$= (k_1 + k_2) \; [\{ \frac{k_1C_{A.0}-k_2C_{B.0}}{k_1 + k_2} \} - X \,] \qquad (3)$$

In which, C_A and C_B are the concentrations of the metal at any time in the solution and on the sorbent, $C_{A,0}$ and $C_{B,0}$ are the initial concentrations of the metal in solution and on sorbent, respectively. According to the mass flow concept, the equilibrium constant, k_e is defined as the ratio of forward to the reverse rate constant. So, at equilibrium conditions :

$$k_e = k_1 / \, k_2 = C_{B,e} \, / \, C_{A,e} \qquad (4)$$

And :

$$dC_A \,/dt = - \, dC_B \,/dt = dX/dt = 0 \qquad (5)$$

From Eqs. 2 and 5 :

$$0 = k_1 \; (C_{A,0} - X_e) - k_2 \; (C_{B,0} + X_e$$

Or:

$$X_e = \frac{k_1C_{A.e}-k_2C_{B.e}}{k_1 + k_2} \qquad (6)$$

In which, $C_{A,e}$ and $C_{B,e}$ are the concentrations of lead at equilibrium, in liquid and on sorbent, respectively; and X_e is the amount of lead conversion at equilibrium.

From Eqs. 3 and 6:

$$dX/dt = (k_1 + k_2)(\, X_e - X) \qquad (7)$$

On integration Eq. 7 gives :

$$\ln \; [X_e \; /(\, X_e - X)] = (k_1 + k_2)^* \; t$$

Or:

$$\ln \; [1 \; - X/ \; X_e \,] = - \; (k_1 + k_2)^* \; t$$

Or:

$$\ln \; [1 \; - \; U(t)] \; = \; - \; k'' \; t \qquad (8)$$

In which,

$$k'' = \text{overall rate constant} = k_1 + k_2 \; = k_1(1 \, + \, 1/k_e) \qquad (9)$$

And, $U(t) = X/X_e$, is called the fractional attainment (Helfferich, 1962). If $\ln \; [1\text{-}U(t)]$ is plotted against time, it should result in a straight line, in case biosorption obeys the underlying assumption of reversible kinetics. The adsorption kinetics of uptake of Pb(II) by *P. ostreatus* linearised according to the above model is presented in Fig. 2. Instead of a single linear profile, the kinetics exhibits an initial linear portion which deviates after 15 minutes. The behaviour of the curve indicates that the reversible kinetics is followed during the initial period. From Fig. 1, it is observed that, a contact time of three hours is sufficient to establish equilibrium, hence an equilibrium contact time of 3 hours was used for subsequent experiments.

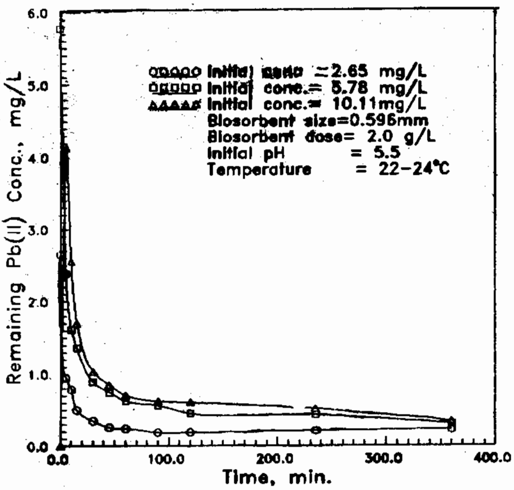

Fig 1. Lead Removal Kinetics Studies.

Sorption isotherm

When an adsorbent is contacted with an adsorbate in a liquid medium, the adsorbate will immediately start moving towards the adsorbent surface. The adsorption process will continue till an apparent equilibrium is established between the liquid phase and on the solid phase. This state is one of dynamic equilibrium, i.e., the migration of the amount of adsorbate from liquid to solid phase and vice versa remains same. At this stage, adsorption should not be thought to be halted. From kinetic experiments, three hours of contact time is found sufficient for the attainment of equilibrium. The sorption isotherms for lead - P. ostreatus systems using different particle sizes are shown in Fig. 3. Experimental data were best fitted to the Freundlich isotherm equation:

$$q_e = k\, C_e^{1/n} \tag{10}$$

$$\log q_e = \log k + 1/n \log C_e \tag{11}$$

where, q_e = the amount of lead sorbed per unit weight of sorbent (mg/g);

C_e = equilibrium concentration of lead in the liquid phase (mg/L);

k = sorption capacity (mg/g); and

n = sorption intensity.

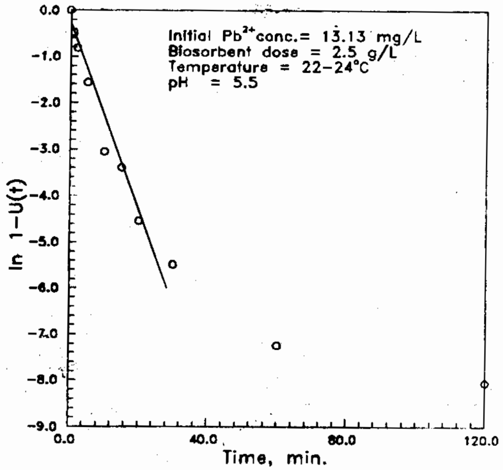

Fig 2. First Order Reversible Kinetic Fit of Lead (II) Biosorption on P. ostreatus.

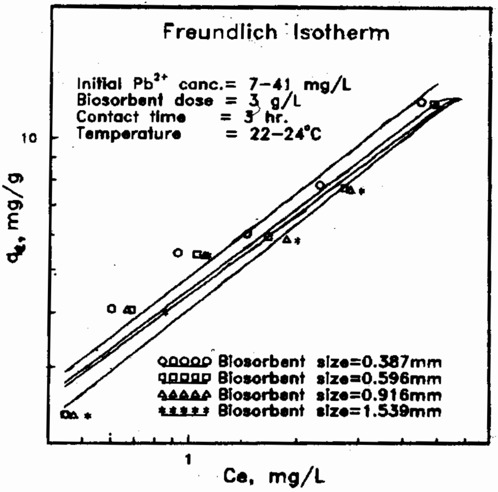

Fig 3. Biosorption Isotherm of Pb(II) onto various sizes of P. ostreatus.

The adsorptive capacity is an important factor in selecting a suitable sorbent system. More the sorptive capacity, better the system since the service time of the sorption column will be more. While increase in sorption with decrease in particle size is obvious, from the figure it is observed that the variation is marginal (4.07 to 4.84 mg/g for an equilibrium effluent concentration of 1 mg/L) making it possible to select the biosorbent over a wide range of particle sizes, without appreciable loss of capacity.

Effect of pH

pH is an important factor, which influences most of the heavy metal removal processes. That is why pH was chosen as master variable by Balistrieri and Murray (1982) in their experiments. In the present investigation, the effect of pH on lead removal was studied in the pH ranges 2.0 to 6.0 and the results are shown in Fig. 4. The result shows that the removal of lead from an aqueous solution is more efficient at higher pH values. The percentage removal of lead at pH 2.0 was found only 6% whereas an increase of 2 units in the pH value resulted an increase in the removal efficiency up to 97%. This sharp increase in the lead removal efficiency can be explained in two ways. First, at low pH, high concentrations of H^+ ions are present in solution which compete for vacant adsorption sites of the biosorbent. This phenomenon was substantiated by the observation of a sharp increase in solution pH after adsorption. Secondly, for hydrolyzable metal ion ($PbOH^+$), there is a critical pH range (often 2 units wide) where the metal removal efficiency increases from a very low level to a maximum value. This pH range is commonly called the adsorption edge. The pH adsorption edge for lead uptake by the biosorbent was found to be from pH 2.0 to 4.0. At higher pH values (more than pH 6.0) lead (II) ions precipitated because of the existence of OH^- ions (Kutsal and Aksu, 1988), so adsorption studies at these pH values couldn't be performed.

Effect of ionic strength

Ionic strength is one of the important factors that influence the aqueous phase equilibrium. Influence of ionic strength adjusted with $NaNO_3$ on the removal of lead onto biosorbent was studied. Result (Fig. 5) indicates that, lead uptake is almost constant upto an ionic strength of 10^{-2} M and for a tenfold increase in the ionic strength (10^{-1} M), uptake was slightly influenced (only 6% reduction). However, for a further tenfold increase in the ionic strength (1M), the lead uptake decreased significantly. Adsorption process is very sensitive to change in concentration of the background electrolyte because the solid phase concentrations depend on the activities of the ions in solution and with the increase of ionic strength, the activity coefficient of ions decreased with the consequent decrease in removal.

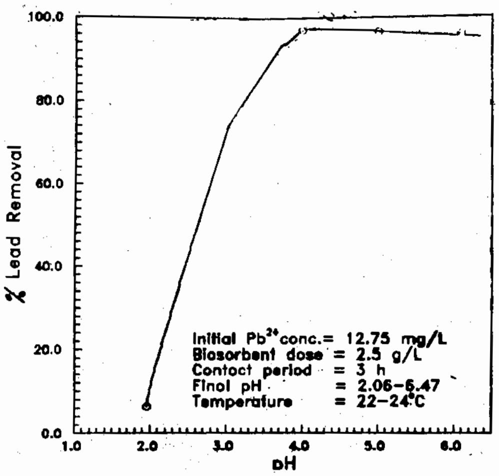

Fig 4. Effect of pH on Lead (II) Sorption.

Effect of EDTA

To investigate the effect of EDTA, which generally occur in metal processing effluents, lead uptake study was conducted in the presence of different concentrations of EDTA (Fig. 6). The result indicates that, even 0.1mM concentration of EDTA inhibited the lead uptake substantially. EDTA is a strong chealating agent making complexes with lead, reducing the lead species favouring adsorption, which resulted in a decrease in lead uptake. Similar effect of EDTA on metal removal was observed in experiments with activated carbon (Reed and Arunachalam, 1994).

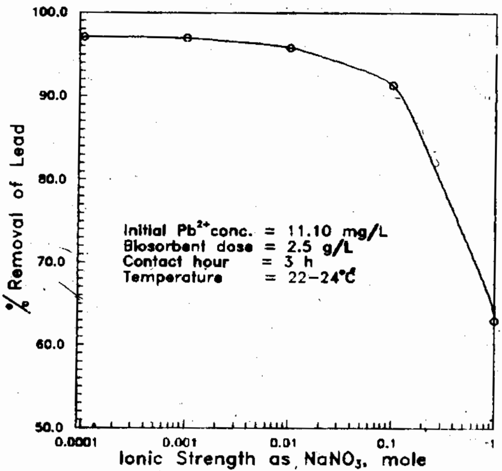

Fig 5. Effect of Ionic Strength on Lead (II) Sorption.

Effect of Calcium and Magnesium

Calcium and magnesium are the common cations present in water which can affect the adsorption of Lead (II). The performance of P. *ostreatus* in the presence of calcium (0 to 100 mg/L) and magnesium (0 to 50 mg/L) on lead removal was studied and the result is presented in Fig. 7. It is observed that the removal efficiency of lead as slightly reduced with increasing concentrations of calcium and magnesium in the solution. In the presence of 100 mg/L of calcium and 50 mg/L of magnesium, the reduction in lead removal efficiency was found to be 3.5% and 3%, respectively. Less reduction in the lead removal was observed in the presence of calcium and magnesium, probably due to selectivity of lead compared to there metal ions, which might be confirmed from the selectivity study of the metals by the biosorbent.

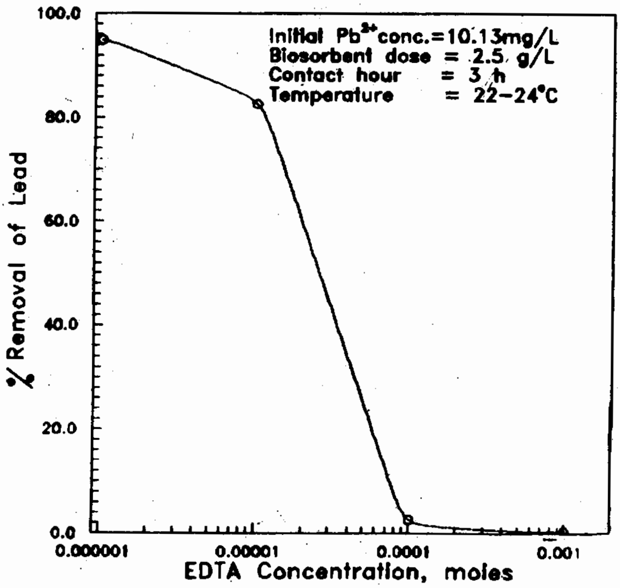

Fig 6. Effect of EDTA on Pb(II) Sorption.

Desorption Study

From the results of desorption study shown in Table 2, it was found that about 99% and 90% desorption of lead (II) was achieved by 0.1N HCL and 0.01N HCL, respectively; whereas a negligible amount of lead (about 8%) was desorbed with distilled water as eluting agent. A contact period of 1 hour was found sufficient to complete the total desorption. More desorption with 0.1N hydrochloric acid indicates the fact that at low pH conditions, protons effectively compete for the active sites, thus releasing the metal ion to the suspending medium, which was also observed during lead adsorption at different pH values .

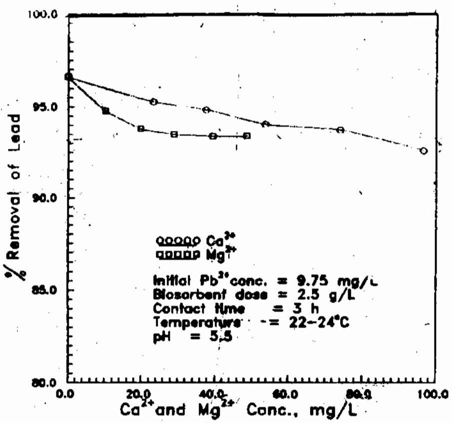

Fig 7. Effect of Ca(II) and Mg(II) on Sorption of Pb(II) onto P. ostreatus.

Table 2. Desorption of Lead (II) by Different Eluting Agent.

Lead (II) Adsorbed, mg/g	Lead (II) Desorbed in D.W, mg/g	Lead (II) Desorbed in 0.01N HCL, mg/g	Lead (II) Desorbed in 0.1N HCL, mg/g
4.882	0.391	4.394	4.853
2.737	0.205	2.491	2.721
1.209	0.103	1.100	1.197

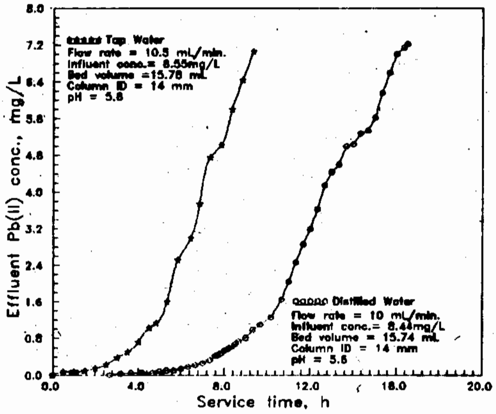

Fig 8. Performance of P. ostreatus columns in lead(II) removal.

Column Studies

Figure 8 indicates the results of the biosorbent column studies for both lead-spiked tap water and distilled water. In the case of tap water, after only 2 h of running, the effluent lead concentration increases and reaches 7.0 mg/L at 9.33 h. This poor efficiency was due to interference of other ions present in the tap water. In case of distilled water, lead has not been detected in the effluent up to 5 h and an effluent concentration of 1 mg/L has been found after 9.5 h. For an initial lead concentration of 8.44 mg/L the ultimate lead removal capacity of the column is calculated to be about 23.6 mg/g which is greater than the ultimate removal capacity (18.7 mg/g) evaluated from the isotherm study. Higher capacity found in the column study shows the better effectiveness of a column for lead removal compared to a batch reactor.

CONCLUSIONS

Based on the present investigation, the following conclusions can be drawn. Locally growing mushroom *P. ostreatus* can be used as an adsorbent for Lead (II) removal from water as both kinetics and sorption capacity are highly favourable. The sorption of Pb(II) onto biosorbent is well described by the Freundlich isotherm. Adsorption of lead is pH-dependent and increases with the increase of pH up to a pH value of 6.0. The presence of high ionic strength (more than 10^{-2} M) and appreciable quantities of a complexing agent like EDTA, significantly reduced the Lead (II) removal. The presence of calcium and magnesium ions in the observed range impedes the lead removal efficiency. Desorption of lead from the loaded biosorbent could be accomplished using dilute acids such as 0.1N HCL. From the results of the down flow column study, the biosorbents P. *ostreatus* was found to be effective in continuous flow system for the separation of lead from both lead spiked distilled water and tap water.

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