

Biosorption of Pb(II) by Nonliving Lichen Biomass of *Cladonia rangiformis* Hoffm

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ABSTRACT: Environmental contamination by toxic metals is a serious problem due to their extreme toxicity towards aquatic life and humans. Biosorption is an alternative technology to conventional processes for the removal of metals. Biosorption of Pb(II) ions on the lichen biomass of *Cladonia rangiformis* Hoffm. is presented in this research. Batch experiments were conducted to study the biosorption properties of lichen biomass for Pb(II) and the effects of initial metal ion concentration, initial pH, biosorbent concentration, stirring speed and contact time on biosorption efficiency. The optimum pH value was found out 5.0 which was the native pH value of solution. Concentrations ranging from 30 to 150 mg/L Pb(II) were studied and the biosorptive removal efficiency of the metal ions was obtained as 99.5%. The experimental biosorption data were fitted to the Freundlich adsorption model and the regression constants were determined at 15°C. The results indicated that the biomass of *Cladonia rangiformis* is a suitable biosorbent for removing Pb(II) from aqueous solutions.

Key words: Biosorption, *Cladonia rangiformis*, Heavy metal, Lead, Wastewater treatment

INTRODUCTION

The presence of heavy metals in the environment is of major concern because of their toxicity and threat to plant and animal life. Anthropogenic sources of heavy metals include process waste streams from metal plating, mining operations and semi-conductor manufacturing operations (Hashim & Chu, 2004). The annual consumption of lead is in the order of 3 million tons, of which 40% is used in the production of electrical accumulators and batteries, 20% is used in gasoline as alkyl additives, 12% in building construction, 6% in cable coatings, 5% in ammunition and 17% in other usages (Volesky, 1990). Lead and its compounds have often been referred to as common and significant pollutants. Lead is also considered among the most toxic heavy metals due to its bioaccumulation potential, inhibition of plant and microbial growth by influencing the pH of the substrate and inactivating the cell enzymes (Al-Subu, 2002; Skountzou *et al.*, 2003). Lead accumulates mainly in bones, brain, kidney and muscles and may cause many serious disorders like anaemia, kidney disease, nervous disorders and sickness even death. Current EPA drinking water standard for lead are 0.05 mg/L, but a level of 0.02 mg/L has been proposed

and is under review (Gupta *et al.*, 2001). Thus, the levels of lead-ion concentrations in wastewater must be controlled and reduced to a set value.

Conventional physico-chemical methods for removing heavy metals from waste streams include chemical reduction, electrochemical treatment, ion exchange, precipitation and evaporative recovery. These processes are generally expensive when the initial heavy metal concentrations are in the range of 10-100 mg/L (Özer & Özer, 2003). Biosorption is an alternative technology to remove heavy metals from dilute aqueous solutions based on the property of certain kinds of inactive and dead biomasses to bind and accumulate these pollutants by different mechanisms such as physical adsorption, complexation, ion exchange and surface micro-precipitation. Natural adsorbents generally used in biosorption studies are inexhaustible, low-cost and non-hazardous materials, which are specially selective for different contaminants and easily disposed by incineration (Veglio & Beolchini, 1997; Pagnanelli *et al.*, 2003a). Different types of biomass have been investigated for biosorption of heavy metal ions. These include fungi Sag *et al.* (1998), yeast

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Bayhan et al. (2001), Özer & Özer (2003), algae Feng & Aldrich (2004), bacteria Pagnanelli et al. (2003b) and inexpensive plant material such as dried leaves Al-Subu (2002), pine bark Machado et al. (2003), sawdust Taty-Costodes et al. (2003), rice husk Ajmal et al. (2003) and lichen biomass Ekmekyapar et al. (2006).

Lichens are plants composed of an alga and a fungus in a symbiotic relationship that often attach to tree bark. Lichens have been widely used as air pollution monitors because of their ability to strongly bind and accumulate many metals. Lichens have also been found to bind metals in a strongly pH dependent manner. Generally optimum binding is observed at a pH of around 5.0. Little binding is seen below pH values of 2.0 for most metal ions, the metal ion-binding properties of lichens have been pointed out that nonliving lichen biomass is able to bond metal-ions to a greater degree than living lichens. This strong metal binding ability of lichen biomass from aqueous solutions would seem to make lichen material an ideal biosorbent for removal of heavy metals (Beck & Ramelow, 1990; Akcin et al., 2001).

The equilibrium of a solute separated between the liquid and solid phases may be described by various models of sorption isotherms. The isotherm represents the equilibrium relationship between the metal uptake by the sorbent and the final metal concentration in the aqueous phase, showing the sorption capacity of the sorbent. Freundlich is one of the widely accepted and easily linearized adsorption isotherm models used in the literature which was proposed.

The Freundlich expression is an empirical equation based on sorption on a heterogeneous surface. The general Freundlich equation is as follows (Freundlich, 1907):

$$q_e = K_f C_e^{1/n} \quad (1)$$

and linearized form of this model is

$$\log q_e = \log K_f + (1/n) \log C_e \quad (2)$$

where intercept, $\log K_f$, is a measure of adsorbent capacity, and the slope, $1/n$, is the intensity of adsorption. q_e and C_e are the amount of metal ion removed and the equilibrium concentration, respectively.

The amount of adsorbed metal ions per gram of biomass was obtained by using the general equation:

$$q_e = [(C_o - C_e) \cdot V] / M \quad (3)$$

where q_e is the amount of metal adsorbed on the biomass (mg/g), C_o is the initial metal ion concentration

in solution (mg/L), C_e is the equilibrium metal ion concentration in solution (mg/L), V is the volume of the medium (L) and M is the amount of the biomass used in the reaction mixture (g).

Lichens are among the most widely used indicators of environmental quality because of their higher capacity for accumulation and retaining a variety of contaminants, particularly heavy metals and radionuclides (Nash & Wirth, 1988). Several studies have been carried out for biomonitoring metal pollution with lichens (Beck & Ramelow, 1990; Al-Shayeb et al., 1995; Reis et al., 1996) but there is lack of information about the sorption ability of lichens. As far as the authors are aware, there is no investigation reported in the literature on the biosorption of Pb(II) by *Cladonia rangiformis*. The lichen biomass is natural, easily available and the use of this low-cost material as biosorbent for dissolved metal ions has been shown to provide economic solution to this global problem. Therefore, this new material was chosen as biosorbent in the study. The aim of the present study was to investigate the use of *Cladonia rangiformis* biomass as a biosorbent for the removal of Pb(II) from an aqueous solution. The optimum biosorption conditions were determined as a function of contact time, pH, initial metal ion concentration, biosorbent concentration and stirring speed.

MATERIALS & METHODS

Biosorbent preparation

The lichen biomass of *Cladonia rangiformis* was used as a biosorbent for the biosorption of Pb(II) ions. Samples of the lichen were collected from the east black sea region of Turkey in July and August of 2003. They were washed with deionized water and inactivated by heating in an oven at 80°C for 48 hours. The inactivated dried lichen biomass was ground and sieved through the following sizes: 75-180, 180-300, 300-425, 425-600 μ m. 180-300 μ m fraction was used in all experiments.

Preparation of stock solution

The stock solution of Pb(II) (500 mg/L) was prepared by dissolving 0.7995 g of $Pb(NO_3)_2$ in deionized water. The required concentrations were prepared from the stock solution by dilution.

Biosorption studies

A known quantity of dried lichen was added to various concentrations (30-150 mg/L) of 100 mL Pb(II) solution in 250 mL Erlenmeyer flasks before the pH adjustment of the Pb(II) solutions. The pH of each solution was adjusted to the required value (2.0, 3.0, 4.0, 5.0 and 5.5) by using HCl and NaOH solutions (0.1 N). The biosorbent concentrations varied from 1 to 10 g/L and the stirring speeds studied were 50, 100, 150 and 200 rpm. The mixture was stirred in a shaker at a

constant speed for 60 min. at the room temperature (15°C). Samples were taken at certain time intervals, filtered by using filter paper (Whatman, 110 mm ϕ and 11 μ m pore size) for removing the suspended biomass and analyzed for residual Pb(II) concentration. The Pb(II) concentration in the supernatant solution was determined using flame atomic absorption spectrophotometer (Shimadzu AA-670) at 283.3 nm (APHA, 1986). Zeta potentials were measured with a zeta-meter (Zeta-Meter 3.0 + 542, USA). All experiments were carried out in a batch stirred system.

RESULTS & DISCUSSION

The mixture of the metal solution and dried lichen were continuously stirred in the shaker at 150 rpm, using 70 mg/L Pb(II) ion concentration for 4h. The metal uptake by dried lichen was very rapid and the equilibrium was reached within 15 min. The biosorption continued until 60. min. with a small increase in removal of metal ions after the equilibrium time as shown in Fig. 1. After this period an increase of residual concentration of metal ion in the solution was observed with time due to starting desorption in the system. This trend suggests that physical adsorption is effective in the bonding mechanism of Pb(II) ions on lichen biomass. Adsorption occurring as a result of van der Waals forces is generally termed “physical” adsorption. Physical adsorption is usually predominant at low temperature and characterized by a relatively low energy of adsorption, that is, the adsorbed molecule is not affixed to a specific site at the surface. This type of adsorption occurring a short time is a reversible reaction and the adsorbed molecule desorbs with changing of adsorbate concentration in solution (Metcalf & Eddy, 1972; Tsezos & Volesky, 1981). Thus 60 min. was fixed as the optimum contact time. pH is one of the most important factor affecting the biosorption process (Veglio & Beolchini, 1997; Pagnanelli *et al.*, 2003b; Selatnia *et al.*, 2004). That can be explained by the strong relation between the biosorption and the number of negative charges at the biomass surface which is itself related to the functional groups. The ionic forms of the metal in solution and the electrical charge of the biomass depend on the solution pH (Selatnia *et al.*, 2004). Batch equilibrium studies were carried out by contacting 0.5 g dried lichen biomass with 100 mL Pb(II) solution. Different initial pH values ranging from 2.0 to 5.5 were studied in order to investigate the effect of pH on the biosorption capacity of lichen biomass for Pb(II). Removal efficiency of Pb(II) increased from 77.2% at pH 2.0 to 95.8% at pH 5.0 (Fig. 2). As the pH increases, the active sites of biosorbent become negatively charged and able to bind positive metal ions in solution. The metal uptake

decreases when the solution pH decreases because of the competition between protons and metal ions for the same active sites on the biosorbent. All the experiments were conducted at pH 5.0 because the maximum metal uptake was obtained in this native pH value of solution. Further increase in pH to 5.5 did not show an increase in biosorption. This can be explained by the decreased solubility of metals at high pH values. At higher pH values (pH>5.5), Pb(II) ions precipitated because of the high concentration of OH⁻ ions in the solution and biosorption studies at these pH values could not be performed. Similar results were also found using *Rhizopus arrhizus* Sag *et al.* (2000), petiolar felt-sheath of palm Iqbal *et al.* (2002) and *Sphaerotilus natans* Pagnanelli *et al.* (2003b).

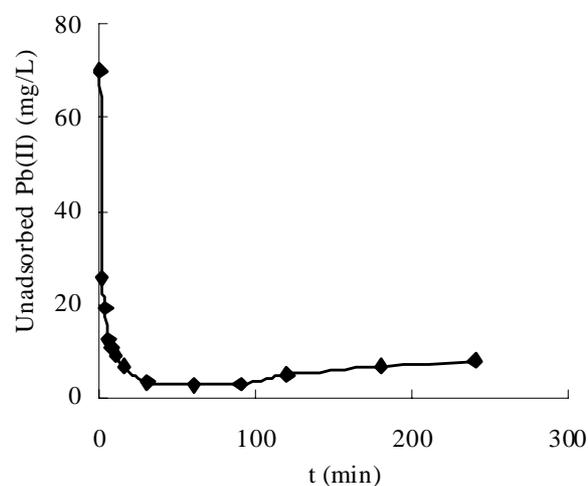


Fig. 1. Effect of contact time on biosorption (Initial metal concentration (C_0) = 70 mg/L, pH = 5.0, temperature (T) = 15 °C, biosorbent concentration (m) = 5 g/L, stirring speed = 150 rpm)

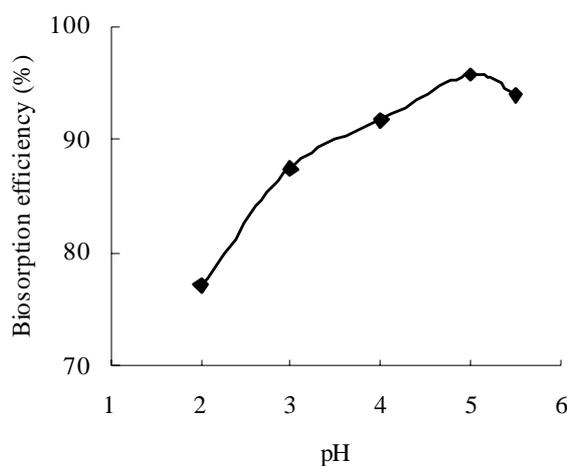


Fig. 2. Effect of pH on biosorption efficiency (C_0 = 70 mg/L, T = 15 °C, m = 5 g/L, stirring speed = 150 rpm, contact time (t) = 60 min)

The electrical potential at the surface of a particle is zeta potential. Zeta potential measurements have been used experimentally to predict optimum pH levels. It can be determined by the measurement of the velocity of particles in the electric field. By measuring how fast particles move across a microscopic grid toward an anode or cathode (positive or negative poles), zeta potential defines the charge on particles in water. The effect of pH on the zeta potential of lichen biomass is illustrated in Fig. 3. Zeta potential values were measured as 0 mV which is the isoelectric pH point at pH 2.0 for deionized water and Pb(II) solution. As the pH increased the negative charge on lichen biomass increased and Pb(II) adsorbed to the negatively charged sites. The maximum zeta potential value ($zP = -40.7$) was obtained at pH 5.0, which corresponded to the maximum sorption efficiency of Pb(II). Therefore, the sorption of Pb(II) to lichen biomass occurred through electrostatic interaction.

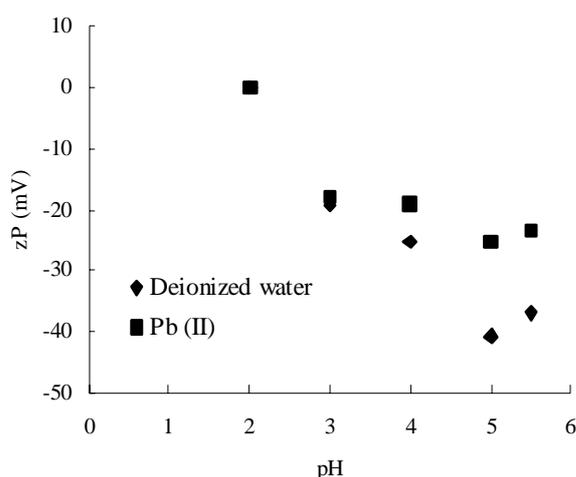


Fig. 3. Relationship between zeta potential and pH ($C_o = 70$ mg/L, $T = 15$ °C, $m = 5$ g/L, stirring speed = 150 rpm, $t = 60$ min)

Effect of initial Pb(II) concentration on biosorption

In order to demonstrate the effect of initial metal ion concentration, biosorption experiments were carried out at different initial Pb(II) concentrations ranging from 30 to 150 mg/L (Fig. 4). The biosorption efficiency decreased with increasing of initial Pb(II) ion concentration, however, the amount of Pb(II) adsorbed on the biomass (mg Pb(II)/g biomass) increased as seen from Table 1. This trend suggests that most of the available sorption sites become occupied at high initial metal ion concentrations. Therefore, more Pb(II) ions were left unadsorbed in solution at higher concentration levels. The study of the isotherms indicates the adsorption capacity of biomass for removal of Pb(II) from the solution at constant conditions. The linearized form of Freundlich

adsorption isotherm presented in Fig. 5 was used to evaluate the biosorption experimental data of Pb(II). The adsorption capacity (K_f) and the adsorption intensity (n) were estimated from the intercept and the slope of $\log q_e$ versus $\log C_e$ graph, respectively and determined as 9.241 and 3.071, respectively. The uptake capacities (q_e) for Pb(II) of lichen biomass and other biosorbents reported in the literature are given in Table 2. The lichen biomass presents a higher value for those various biosorbents.

The effect of biosorbent concentration on biosorption efficiency is presented in Fig. 6. Pb(II) biosorption on lichen biomass was studied at various biosorbent concentrations ranging from 1 to 10 g/L. The increase in biosorbent concentration from 1 to 5 g/L resulted in an increase in removal of Pb(II) ions because of the availability of more binding sites for complexation of Pb(II) ions. Pb(II) Biosorption efficiency (%) Biosorption efficiency (%) removal efficiency was the maximum at 5 g/L of biosorbent. But further increases in biosorbent concentration decreased the maximum removal of Pb(II) ions. This can be explained by forming aggregates during biosorption which takes place at high biomass concentrations causing a decrease of the effective adsorption area. An increase in biosorbent concentration generally increases the adsorbed metal ion concentration because of an increasing adsorption surface area. But in several studies reported in the literature a decrease was found in the biosorption capacity of biomass (Aksu *et al.*, 1990; Esposito *et al.*, 2001; Selatnia *et al.*, 2004). The researchers implied that a partial cell aggregation taking place at high biomass concentrations caused a decrease of active sites. Various reasons including pH, ionic strength, temperature, metal ion in solution and biomass concentration have been suggested to explain the decreased adsorption capacity at increasing biomass (Esposito *et al.*, 2001).

Biosorption studies of Pb(II) with lichen biomass were carried out in a shaker working at pH 5.0 using 70 mg/L Pb(II) solution. The stirring speed varied from 50 to 200 rpm. Samples were taken at certain time intervals and analysed for Pb(II) ion concentration in solution; the results are presented in Fig. 7. The maximum biosorption efficiency was obtained at 150 rpm. The increase in stirring speed from 50 to 150 rpm resulted in an increase in removal efficiency of Pb(II) ion. The rate of adsorption is controlled by either film diffusion or pore diffusion, depending on the amount of agitation in the system. If relatively little agitation occurs between the particle and fluid, the surface film of liquid around the particle will be thick and film diffusion will likely be the rate-limiting step. The mass transfer rate

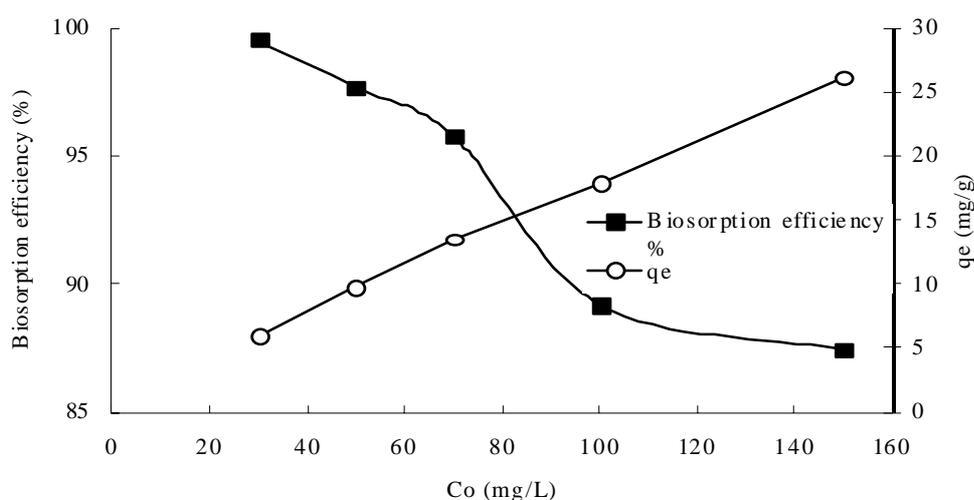


Fig. 4. Effect of initial Pb(II) concentration on Pb(II) uptake ($q_e = \text{mg Pb(II)/g biomass}$) and biosorption efficiency (pH = 5.0, $T = 15^\circ\text{C}$, $m = 5 \text{ g/L}$, stirring speed = 150 rpm, $t = 60 \text{ min}$)

Table 1. Effect of initial Pb(II) concentration on biosorption (pH = 5.0, $T = 15^\circ\text{C}$, $m = 5 \text{ g/L}$, stirring speed = 150 rpm, $t = 60 \text{ min}$)

$C_o, \text{mg/L}$	$C_e, \text{mg/L}$	Adsorbed Pb(II), mg/L	$q_e, \text{mg/g}$
30	0.153	29.847	5.969
50	1.161	48.839	9.767
70	2.912	67.088	13.417
100	10.77	89.23	17.846
150	18.98	131.02	26.204

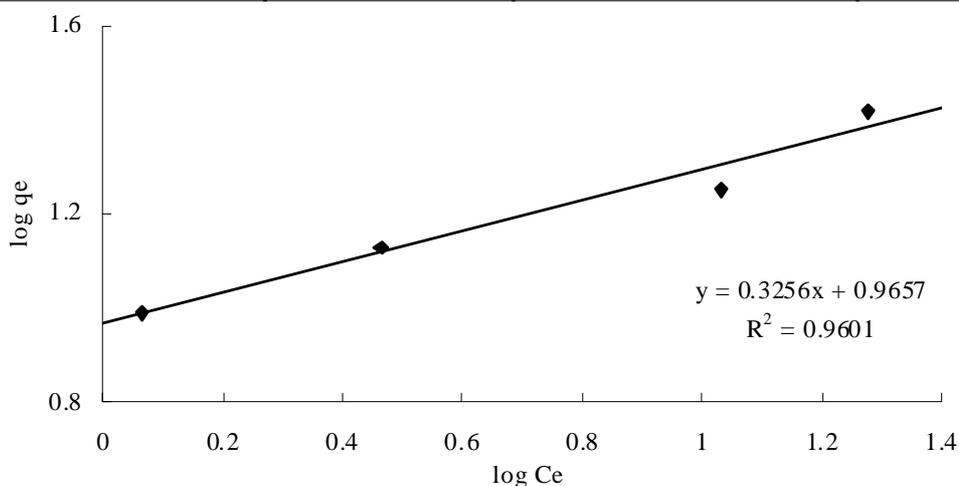


Fig. 5. Freundlich adsorption isotherm for Pb(II) by lichen biomass (pH = 5.0, $T = 15^\circ\text{C}$, $m = 5 \text{ g/L}$, stirring speed = 150 rpm, $t = 60 \text{ min}$)

Table 2. Uptake capacities for Pb(II) of various biosorbents reported in the literature

Adsorbent	$q_e, \text{mg/g}$
<i>Saccharomyces cerevisiae</i> [Veglio & Beolchini, 1997]	2.7
Sawdust [Bulut & Tez, 2003]	4.48
Cone biomass [Ucun <i>et al.</i> , 2003]	11.38
Petiole felt-sheath of palm [Iqbal <i>et al.</i> , 2002]	11.4
Olive pomace [Pagnanelli <i>et al.</i> , 2003a]	15.732
<i>Penicillium digitatum</i> [Sag <i>et al.</i> , 2000]	18.65
Olive mill [Gharaibeh <i>et al.</i> , 1998]	21.56
<i>Rhizopus arrhizus</i> [Sag <i>et al.</i> , 2000]	22.94
<i>Cladonia rangiformis</i> (this study)	26.204
<i>Penicillium chrysogenum</i> [Niu <i>et al.</i> , 1993]	116.0

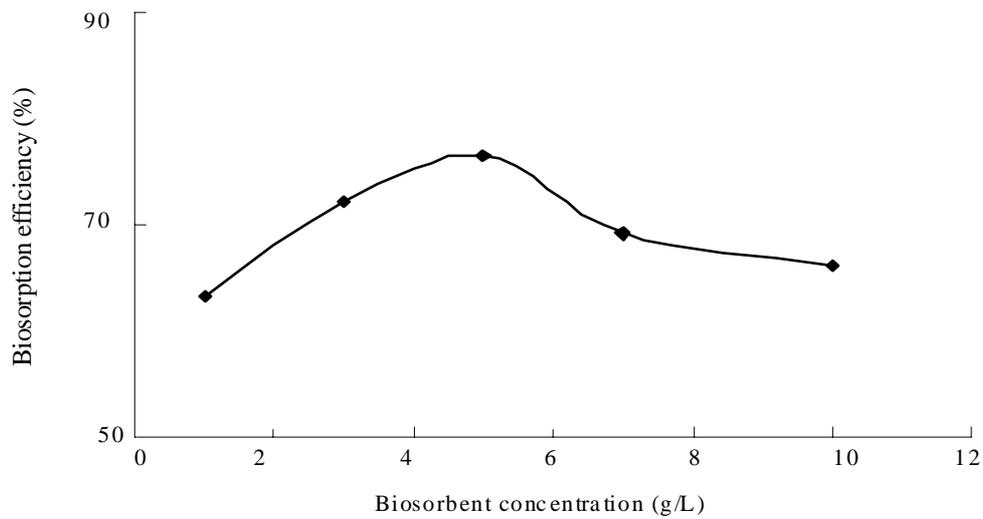


Fig. 6. Effect of biosorbent concentration ($C_o = 70$ mg/L, pH = 5.0, $T = 15$ °C, stirring speed = 150 rpm, $t = 60$ min)

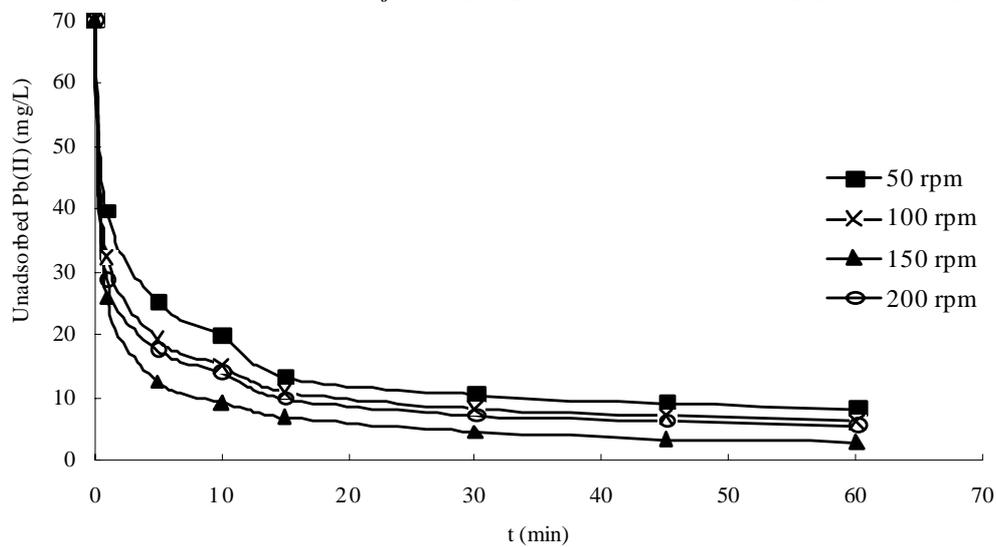


Fig. 7. Effect of stirring speed on biosorption ($C_o = 70$ mg/L, pH = 5.0, $T = 15$ °C, $m = 5$ g/L, $t = 60$ min)

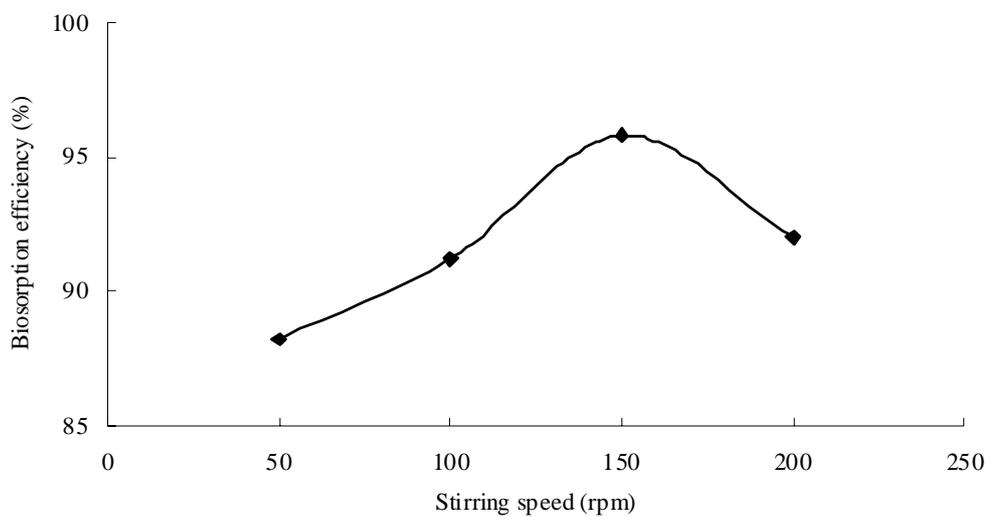


Fig. 8. Effect of stirring speed on biosorption efficiency ($C_o = 70$ mg/L, pH = 5.0, $T = 15$ °C, $m = 5$ g/L, $t = 60$ min)

increases with the increase in stirring speed. The boundary layer thickness decreases with increased stirring speed which results in a reduction in surface film resistance (Benefield *et al.*, 1982). Therefore, the metal ions adsorbed to the biosorbent surface more easily. If adequate mixing is provided, the rate of film diffusion will increase to the point that pore diffusion becomes the rate-limiting step. Pore diffusion is generally rate-limiting for batch-type contacting systems which provide a high degree of agitation (Benefield *et al.*, 1982). For this reason, further increase in stirring speed did not show an increase in the biosorption efficiency (Fig. 8). At 200 rpm, the biosorption efficiency was found to be slightly lower than that at 150 rpm. This result indicated that the contact between solid and liquid was more effective at moderate speed (150 rpm). Similar results were reported in the literature (Prakasham *et al.*, 1999; Ucin *et al.*, 2003; Selatnia *et al.*, 2004).

CONCLUSION

The removal of Pb(II) ions from aqueous solution by lichen biomass of *Cladonia rangiformis* was studied in a batch system. The experimental run was observed during 4 h. and the contact time was determined as 60 min. The biosorption was rapid and the equilibrium was reached within 15 min. This rapid metal sorption is highly desirable for successful deployment of the biosorbent for a practical application in industrial wastewater treatment. The biomass exhibited high biosorption capacity. The results showed that pH and initial metal concentration significantly affected the biosorption performance. The maximum biosorption efficiency was 99.5% at $C_0=30$ mg/L, pH 5.0 and 150 rpm for a 5 g/L lichen biomass concentration. In addition, the biosorption efficiency was greater than approximately 80% at all pH values ranging from 2 to 5.5. The biosorption equilibrium data fitted well to the Freundlich isotherm. *Cladonia rangiformis* can be successfully used as a biosorbent for the biosorption of Pb(II) ions. This natural material is non-hazardous, easily available and economic for treatment of industrial wastewater.

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