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Azadirachata indicum (Neem): An Effective Biosorbent for the Removal of Lead (II) from Aqueous Solutions

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Abstract Laboratory batch experiments with *Azadirachata indicum* indicated that this population had an excellent ability to bind lead (II) from its aqueous solution. The experiments carried out examined pH, biomass quantity, time of contact, and temperature dependency. Under optimum conditions, the removal of lead (II) was found to be around 95%. Column experiments were performed to examine the binding of lead (II) to silica-immobilized biomass under flow conditions. During this, a slight decrease in the pH of the effluents was also observed, implying an ion-exchange mechanism for metal binding.

Keywords *Azidarachata indicum* · Lead (II) · Aqueous solution · Silica-immobilized biomass · Biosorption

Wastewater treatment is a burning issue for the modern industrialized world. These waters contain certain hazardous compounds, mainly heavy metals. Mine drainage, metal industries, refining, electroplating, dye and leather industries, domestic effluents, landfill leachate, agricultural runoff, and acid rain are the sources of waste waters containing heavy metals (Aksu and Kutsal, 1990). Among the toxic heavy metals, mercury, lead and cadmium are known as the big three, due to their major impact on the environment (Volesky, 1994). Various techniques already in use include reverse osmosis, electrodialysis, ultrafiltration, ion exchange, precipitation, and phytoremediation; the main purpose of these technologies is to get rid of heavy metals. Application of such methods, however, is sometimes restricted because of technical or economical constraints (Bossrez et al., 1997; Yu and Kaewsarn, 1999) especially when the heavy-metal ions are in concentrations of 1–100 mg per litre (Volesky, 1990). This has led scientists to investigate low-cost processes and techniques and has resulted in the discovery of techniques such as biosorption and biotechnology. Biosorption utilizes diverse biological materials for metal uptake or binding. This method is advantageous and non-polluting and can be highly selective, more efficient, easy to operate and hence cost-effective (Puranik and Pakniker, 1999). Bacteria (Corder and Reeves, 1994) and materials from algae (Seki and Suzuki, 1998), fungi (Fayuan et al., 2005), yeasts (Bakkaloglu et al., 1998), seaweeds (Vijayraghavan et al., 2005) and higher plants (Horsfall et al., 2003; Gardea-Torresdey et al., 1999) have been proved to be potential metal biosorbents. Metal uptake during this process is believed to be through sorption involving the functional groups associated with proteins, lipids, carbohydrates, lignins, and other biopolymers present in the cell and cell wall (Al-Asheh and Duvnjak, 1999; Wang, 2002). Fundamental information on mechanisms and potential applications of biotechnology have been discussed by various researchers (Mullen et al., 1989; Ashly and Roach, 1990; Francis, 1990; Brierley, 1991; Ehrlich, 1994).

Among heavy metals, lead has a special contribution towards the toxicity caused in the living system. The principal vulnerable areas are the haemopoietic system, leading to anaemia, and the nervous system, leading to brain damage. A high proportion of lead in the environment in many parts of the world comes from vehicle emissions and industrial waste (Baird, 1995).

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In our laboratories, we are investigating the potential of various types of local biomass to remove polluting metals from industrial effluent. Specifically we report herein our findings on the use of the biomass of a local plant, *Azadirachata indicum*, found abundantly in many parts of Asia. It is a large evergreen tree reaching about 15 m in height. Its fruit contains alkaloids, tannins, and aromatic acids. Its leaves are useful in many skin diseases and are externally applied to wounds and ulcers in the form of poultices, ointments, and linaments. The juice of the leaves is used to treat jaundice with much claimed success (Bakhshi et al., 1999). In order to help understand the metal binding mechanism, batch laboratory experiments were performed to study the effect of different parameters.

Materials and Methods

Stems of *Azadirachata indicum* were collected from Bahawalpur area, Pakistan. The pre-washed stem/bark material was dried in the shade and ground to 100 mesh. A standard stock solution of lead (1,000 mg/L) was prepared by dissolving lead (II) nitrate (AR grade, Merck) in deionized water. Standard concentrations of lead (II) having 5, 10, 15, and 20 mg of lead (II) per litre of solution were prepared and analyzed to draw a calibration curve. The metal concentration was determined by the use of an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 100) using the lamp at specific conditions. All the glassware was washed with HCl (0.1 M) before and after each experiment to avoid binding of the metal.

Five hundred milligrams of biomass was taken each in seven different measuring flasks (100 mL). Standard lead (II) solution (10 mg/L) was added to each flask and agitated using a shaker for times of 5, 10, 20, 30, 40, 50, and 60 min. The solutions were filtered and analyzed by the instrument.

Varying quantities of biomass (500, 1,000, 1,500 and 2,000 mg) were taken in four different flasks. Standard lead (II) solution (10 mg/L) was added to each and shaken for 10 minutes. Filtered solutions were analyzed for metal determination.

Standard lead (II) solution (10 mg/L) was prepared and taken in seven different flasks. The pH of the solution in individual flasks was adjusted to 3, 4, 5, 6, 7, 8, 9, and 10 using HCl (0.01M) or NaOH (0.01 M) as per requirement. One thousand five hundred milligrams of biomass was added to each and agitated for 10 min. The solutions were filtered and analyzed for metal concentration.

In three different flasks, 1,500 mg biomass was added separately. Standard lead (II) solution (10 mg/L) at pH 5 was added to each one and shaken for 10 min at room temperature 30, 40, and 50°C. The filtered solutions were analyzed to determine the metal concentration.

Biomass from the above temperature studies (at 30° C) with adsorbed lead (II), now referred to as the spent biomass, was exposed to 2 mL of HCl (0.1 M), equilibrated by rocking for five minutes and then centrifuged. Supernatants were collected for analysis and diluted if necessary to stay within the calibration range. Biomass was then exposed to 2 mL of HCl (1 M) to strip any remaining metal and equilibrated by rocking for five minutes. Again after centrifugation, the supernatants were analyzed. The second step of the whole process was repeated.

The method for immobilization of biosorbent within a polysilicate matrix was similar to that already reported (Ke and Rayson, 1993). A 5 g sample of biomass was washed twice by vortexing the sample with water and was centrifuged for 5 min at 3,000 rpm. This step removed soluble material and debris. Next, 75 mL of sulphuric acid (5%) was mixed with enough sodium silicate (6%) solution to raise the pH to 2. At pH 2, washed biomass (5 g) was added to the silica solution and stirred for 15 minutes. The pH was then raised slowly by addition of sodium silicate (6%) to reach a final value of 7. The polymer gel was washed with water until the addition of two drops of barium chloride solution produced no white precipitates of barium sulphate. The polymer gel with immobilized biomass was dried overnight at 60°C and then ground using a mortar and pestle and sieved to a 100 mesh size.

One bed volume equals the volume of the immobilized biomass inside the column. Six milliliters of immobilized *Azadirachata indicum* was used in the column. The column was washed with 10 bed volumes of sodium acetate (0.01 M) buffer at pH 5 and the effluent pH was checked to ensure that the column was at optimal binding pH (i.e., 5). A flow rate of 1 mL/min was used to pass 120 bed volumes of lead (II) solution (10 mg/L) in sodium acetate solution (0.01 M) at a pH of 5. Each bed volume was collected and analyzed. The pH of the eluted bed volumes was also monitored.

To remove the bound lead (II), HCl (0.01 M) was passed at a flow rate of 1 mL/min. Each bed volume was collected and analyzed.

All the experiments were performed in triplicate and the standard deviation was found to be less than 0.5 for all. The percentage removal of metal was found by applying the formula

$$\% \text{ removal} = [\text{metal bound}/\text{original conc. of metal}] \times 100$$

Results and Discussion

The calibration curve for lead (II) was quite linear and reproducible, obeying the Beer-Lambert law. The experiments were performed to determine how long it would take the lead (II) ions to bind to the biomass. These showed that increasing time of contact of the metal and biomass increased the metal binding. However, after a short period

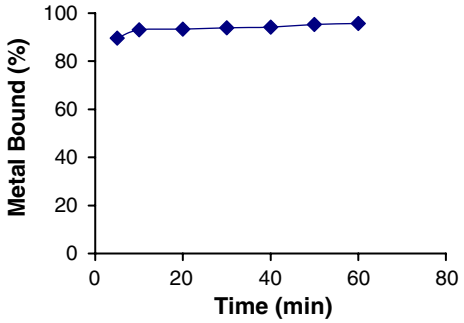

Fig. 1 Variation of metal binding with time

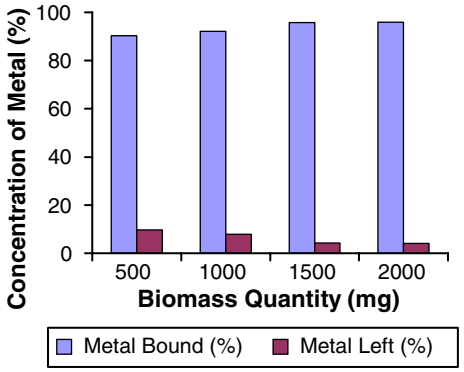

Fig. 2 Effect of dose of biomass on meal removal

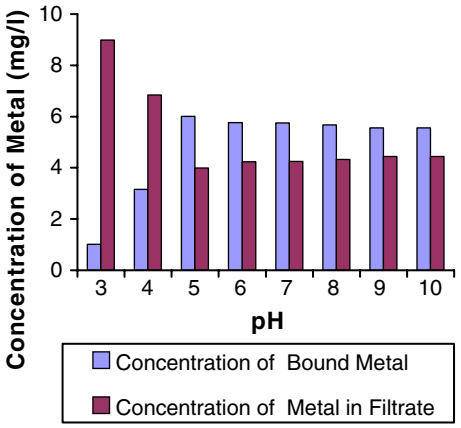

Fig. 3 Influence of the pH of the solution

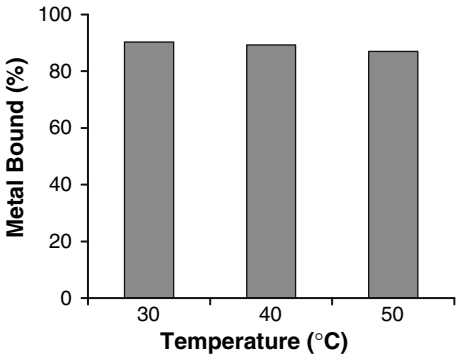

Fig. 4 Effect of the temperature of metal-binding medium on metal sorption

of time there was no significant increase in the level of bound metal as is clear from Fig. 1. The shape of the curve showed that the equilibrium was established within first 10 minutes. Only a 2.6% increase was observed in the metal binding in next 50 minutes. This showed that the binding of lead (II) was relatively stable. The optimum time of contact, thus determined, was 10 minutes.

A comparison of sorption performance of different quantities of biomass was carried out. Although there was decrease in the concentration of metal in the filtrate, the rate of metal binding was not as fast as the increase in the biomass quantity (Fig. 2). This revealed that, for a 10 mg/L solution of lead (II), 1,500 mg biomass showed the best results.

The dependence of metal binding on pH value is depicted in Fig. 3. Under similar conditions of metal concentration (10 mg/L), contact time (10 minutes), and biomass quantity (1,500 mg), the maximum metal binding was achieved at pH 5. At pH values higher than 8, lead (II) ions started to precipitate out of the solution. The biosorption capacity is sensitive to pH (Simine et al., 1998). The pH has been reported to be the most important factor of all. Ion uptake and removal capacity were shown to increase with pH but the upper limit of working pH was limited by hydroxide precipitation (Geddie and Sutherland, 1993). The results are in good agreement with the previous ones, showing that lead (II) has maximum binding at pH 5 (Iqbal et al., 2002).

The trend in the pH binding suggests that carboxyl groups may play a role in metal binding by the biomass. The ionization constants for various carboxyl groups have been reported to be around 3–4 (Hunt, 1986; Segal, 1976). Free carboxyl groups are protonated at pH values lower than 3 and reduce any metal binding. At pH values greater than 4, the carboxyl groups are deprotonated and attract positively charged metal ions. At still higher pHs, the metal ions start to precipitate out due to hydroxide formation (hydroxide ions being present in the solution).

The shape of the graph also suggests that, by decreasing the pH of the biomass-metal system, the bound metal may be recovered and the biomass may be recycled.

Under similar conditions (pH, agitation time, and biomass quantity), the temperature affected the sorption in such a way that, with increase in temperature,the metal binding somewhat decreased. Figure 4 shows that, at room temperature (30°C), most of the lead (II) is removed from its aqueous solution.

These studies reveal that lead (II) removal meets a 95% level by the biomass under investigation (Figs. 1–4).

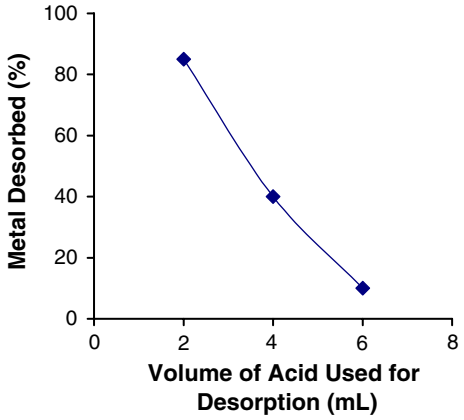

Fig. 5 Desorption of bound lead (II) ions by decreasing the pH of medium

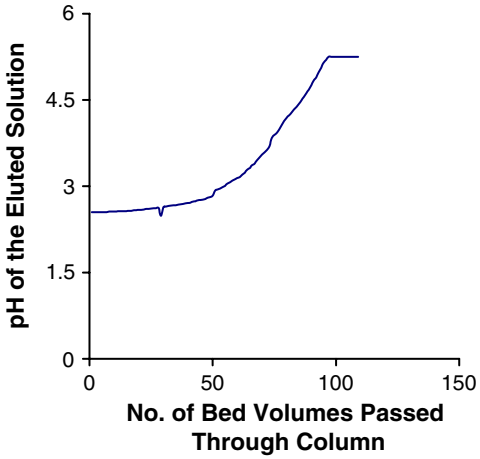

Fig. 7 Change in pH of the effluent in the column experiments

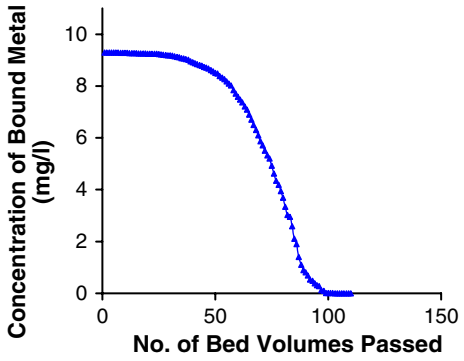

Fig. 6 A continuous flow study of sorption of lead by *Azadirachata indicum*

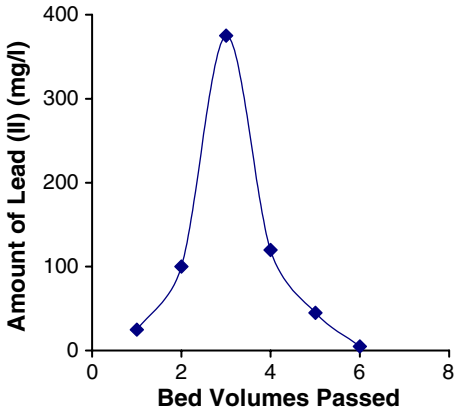

Fig. 8 Recovery of metal from metal-saturated biomass

To confirm the hypothesis that a decrease in pH recovers the bound metal, the spent biomass, from the temperature studies (at 30°C), was subjected to dilute acid (HCl 0.1 M). The results showed that the hypothesis worked and the acid caused the recovery of the bound metal. These desorption studies are represented in Fig. 5.

Instead of the removal of lead (II) ions from aqueous solutions by the batch experiments, it would be more practical if the biomass could be packed into a column so that the aqueous solution could simply be passed through it. When the biomass is simply packed, it clumps and flow rates are quickly reduced. Immobilizing the biomass solves this problem. Thus a polysilicate matrix support material was used for this purpose. This would give the physical properties of the polymer resin as well as binding properties of the biomass. Figure 6 shows the amount of lead (II) ions that remained in the column after a lead (II) solution (10 mg/L) at pH 5.0 was passed through a column of immobilized *Azadirachata indicum*. It is clear that most of lead (II) was retained in the column until the bed volume 40 was reached. After this point trace amounts of lead (II) emerged in the effluent. This may be due to saturation of the binding sites. At bed volume 90, the column was able to bind very little lead (II).

Since all of the soluble components of the biomass were eliminated in prior working, the binding must be due to the biomass alone. As the cells were inactivated by drying them, the rapid binding of the lead (II) ions may be due to the functional groups located on the cell wall and not due to any cellular process.

The pH of each bed volume was also monitored after passing through the column (Fig. 7). The observations showed that the pH was lowered. With the passage of more solution, the pH started to increase to the pH of the solution. The decrease in the pH suggested an ion-exchange mechanism for metal binding, i.e., exchange of biomass functional-group (carboxyl groups) protons with the metal ions present in the solution.

To desorb the bound metal, dilute acid (HCl, 0.01M) was passed through the column. As discussed earlier, the metal was desorbed and quantified. The amount of the metal thus desorbed is shown in Fig. 8. The same column

was again used to remove lead (II) from solution to determine any adverse effect of acid on the immobilized biomass. The results were promising in the sense that the biomass even after 10 cycles of removal and recovery, was still able to bind lead (II) from solution.

The series of experiments carried out suggests that *Azadirachata indicum* biomass possesses a remarkable ability to take up the hazardous metal ion lead (II) from aqueous solution. Immobilized biomass in a column was successful as a biofilter in removing and recovering lead (II), ions and the column was also reusable. *Azadirachata indicum* is easily available, inexpensive, and is also a practicable material for this purpose.

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