ISSN: 0128-7680
Pertanika J. Sci. & Technol. **17 (1)**: 191 – 199 (2009)

Biosorption of Pb (II) Ions by Immobilized Cells of *Pycnoporus sanguineus* in a Packed Bed Column

Mashitah Mat Don*, Yus Azila Yahaya and Subhash Bhatia
School of Chemical Engineering, Universiti Sains Malaysia,
Engineering Campus, Seri Ampangan,
14300 Nibong Tebal, Penang, Malaysia
**E-mail: chmashitah@eng.usm.my*

ABSTRACT

The removal of heavy metals like lead, copper and cadmium from wastewater streams is an important environmental issue. The capability of immobilized *Pycnoporus sanguineus* (*P. sanguineus*), a white-rot macrofungi to remove heavy metals from aqueous solution in a packed bed column was investigated. Lead (Pb (II)) biosorption by immobilized cells of *P. sanguineus* was investigated in a packed bed column. The experiments were carried out by considering the effect of bed height (5-13 cm), flow rate (4-12 ml min^{-1}) and initial lead (II) concentration (50-300 mg L^{-1}). The breakthrough profiles showed that the saturation of metal ions was achieved faster for 5 cm bed height and 12 ml min^{-1} influent flow rate. However, the breakthrough time decreased as the initial metal concentration increased from 50 to 300 mg L^{-1}. The column was regenerated using 0.1M HCl solution and biosorption-desorption studies were carried out for 2 cycles. The results showed that the breakthrough time decreased as the number of cycle was proceeded.

Keywords: Biosorption, breakthrough curve, column, desorption, fungus, immobilization, lead, *Pycnoporus sanguineus*

ABBREVIATIONS

C_o	initial concentration (mg L^{-1})
C	outlet concentration (mg L^{-1})
F	flow rate (ml min^{-1})
H	bed height (cm)
M	mass of biosorbent (g)
m_{ad}	total adsorbed Pb (II) (mg)
m_d	metal mass desorbed (mg)
m_{total}	total amount of metal feed to the column (mg)
Q	metals uptake capacity (mg g^{-1})
R	total metal removal (%)
t	time (min)
t_b	breakthrough time (hr)
t_e	exhaustion time (hr)

Received: 28 January 2008
Accepted: 20 May 2008
*Corresponding Author

INTRODUCTION

To date, various treatment technologies have been introduced for an efficient removal of heavy metals from an industrial effluent. One of these technologies is biosorption process, which utilizes biological materials including fungi, algae, bacteria and yeast, to accumulate metal ions from wastewater (Arica *et al.*, 2001). Conventional methods such as chemical precipitation, electrochemical treatment, membrane technology and ion exchange processes may be inefficient and expensive when operated at low metal concentration (1-100 mg L^{-1}) (Cruz *et al.*, 2004; Malkoc and Nuhoglu, 2006). Some of these treatments produced toxic sludge which may cause further disposal problem. Since these biological materials are abundant and capable to adsorb metal ions, biosorption process has emerged as an alternative method used in removing heavy metal over conventional methods (Cordero *et al.*, 2004; Cruz *et al.*, 2004). Application of fungi, as a biosorbent in heavy metals removal, has received a great attention (Mittar *et al.*, 1992; Arica *et al.*, 2003).

Many fungal species, such as *Aspergillus niger*, *Rhizopus* sp., *Saccharomyces* spp., *Mucor* sp and *Phanerochaete crysosporium,* have extensively been studied as a potential biosorbent in metal ions removal (Kapoor and Viraraghavan, 1997; Say *et al.*, 2001; Kim *et al.*, 2003; Yan and Viraraghavan, 2003). For an efficient use of biosorbents in heavy metal removal in an industrial operation, these free fungal cells were immobilized in carbohydrate-based polymers including alginate, chitin, chitosan and carboxymethyl cellulose (Jianlong *et al.*, 2000; Arica *et al.*, 2003). These immobilized cells offer several advantages, including minimal clogging in continuous systems (Ting and Sun, 2000; Arica *et al.*, 2001; Bayramoglu *et al.*, 2003), which is easy to separate from the reaction system and can be regenerated and reused (Arica *et al.*, 2001; Annadurai *et al.*, 2007).

Mashitah *et al.* (1999) reported that the non-living biomass, known as *Pycnoporus sanguineus* (*P. sanguineus*), or white rot fungi, is one of the potential biosorbent for Pb (II), Cu (II) and Cd (II) biosorption. However, the utilization of *P. sanguineus* cells, in an immobilized system, is lessly reported. Therefore, this study was carried out to determine the potential of the live immobilized cells of *P. sanguineus* to adsorb Pb (II) ions in a packed bed column.

MATERIALS AND METHODS

Microorganism, Medium and Growth Conditions

P. sanguineus, which is capable of adsorbing heavy metals was obtained from the Forest Research Institute Malaysia (FRIM), located in Kepong, Selangor. The culture was maintained by a weekly transfer on malt extract agar slant, incubated at 30°C for 6 days, after which they were stored at 4°C until required.

The composition of the medium used comprised of glucose 20 g L^{-1}, yeast extract 10g L^{-1} and malt extract 10 g L^{-1}. The pH of the medium was adjusted to pH 9, prior to autoclaving at 121°C (1.5 bar) for 15 minutes.

Cell suspension was prepared by inoculating a stock culture of *P. sanguineus* onto the malt extract agar plates, and incubated at 27°C for 6 days. The formed mycelium mat was scraped using a sterile blade and mixed with 10 ml sterile Tween 20 solution prior putting it into a sterile sampling bottle (100 ml). The sampling bottle was then vortexed for 3 minutes so that the mycelium would evenly be distributed in the liquid.

15 ml of the cell suspension was inoculated into an Erlenmeyer flask containing 135 ml of the production medium. The flask was incubated in a rotary shaker at 30°C, 150 rpm for 66 hr. The sample was then harvested and centrifuged at 3500 rpm for 4 minutes.

The Preparation of Immobilized Cells

The sodium alginate beads were prepared by dropping a mixture of sodium alginate solution and *P. sanguineus* cells into 2% (w/v) $CaCl_2$ solution under magnetic stirring (slow) at room temperature. The beads were stirred in this solution for 30 minutes. Successively, they were collected by filtration, washed three times with sterile deionized water and stored in Tris-HCl buffer pH 7 at 4°C until used.

The Preparation of Metal Ions

The metal solutions were prepared by diluting 1000 mg L^{-1} of Pb $(NO_3)_2$ solutions with deionized water to a desired concentration ranged between 50 to 300 mg L^{-1}. For each of the solutions, the initial concentrations of the metals and samples after the biosorption treatment, were determined using an Atomic Absorption Spectrometer (Model Shimadzu AA 6650).

The Biosorption Procedures

The biosorption studies were performed in a jacketed glass column (length 60 cm, i.d. 4 cm) at room temperature. The immobilized beads were packed into it using a wet packing technique. The bed was supported and closed using glass wool plugs to ensure a good liquid distribution at the top and bottom of the column. *Fig. 1* below shows the experimental set up used in the present study. The experiments were carried out to study the effects of the following variables, (i) bed height (5-13 cm), (ii) flow rate (4-12 ml min^{-1}) and (iii) initial Pb (II) concentration (50-300 mg mL^{-1}).

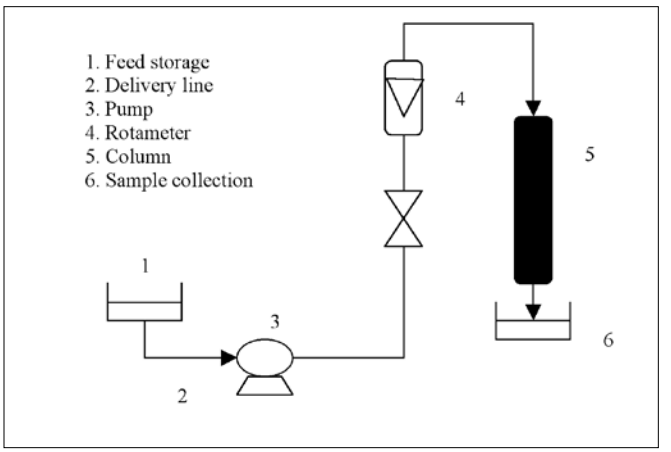

Fig. 1: Experimental set up for the biosorption studies in fixed bed column

In a typical experiment, a known Pb (II) ion concentration feed (100 mg L^{-1}) was pumped at a fixed flow rate into the column with a known bed height. Samples were collected periodically and analyzed for the Pb (II) concentration, using the Atomic Absorption Spectrometer (AAS) until saturation was reached in the column. The breakthrough curve was obtained by plotting C/C_o against time or outlet concentration, and C against time (t). The operation of the column was stopped when the effluent Pb (II) concentration

exceeded a value of 99.5% of the initial feed concentration. The total quantity of the metal mass, biosorbed in the column (m_{ad}), was calculated from the area above the breakthrough curve (outlet concentration, C versus time, t) and multiplied by the flow rate. Dividing the metal mass (m_{ad}) by the mass of biosorbent (M) resulted in the metal uptake capacity (Q) (Volesky *et al.*, 2003; Padmesh *et al.*, 2005). The breakthrough time (t_b) was the time at which metal concentration in the effluent reached 0.01% of the initial feed concentration and exhaustion time (t_e), and at which metal concentration exceeded 99.5 % of the initial feed concentration, respectively.

The total adsorbed Pb (II), m_{ad} (mg) in the column for a given Pb (II) concentration and the flow rate, is calculated as:

$$m_{ad} = \frac{F}{1000}\int_{t=0}^{t=t_{total}} C_{ad}\, dt \tag{1}$$

Meanwhile, the total amount of the metal feed to the column (m_{total}; mg) is:

$$m_{total} = \frac{C_o F t_e}{1000} \tag{2}$$

The mass transfer zone can be calculated using the following Eq. (3):

$$\Delta t = t_e - t_b \tag{3}$$

The total metal removal, R (%) with respect to flow volume is given as:

Total metal removal,
$$R(\%) = \frac{m_{ad}}{m_{total}} \times 100 \tag{4}$$

The metal mass desorbed, m_d (mg) can be calculated from the elution curve (C versus t) and the elution efficiency is given as:

$$E(\%) = \frac{m_d}{m_{ad}} \times 100 \tag{5}$$

Loaded biosorbents with metal ions were regenerated with 0.1 M HCl by pumping it in a down-flow operation of the column. After the regeneration, the biosorption studies were carried out again. This biosorption-regeneration was repeated two times in order to investigate the biosorption capacity of the immobilized cells. The continuous experiments were conducted at room temperature (30°C).

RESULTS AND DISCUSSION

The Effect of the Bed Height

Fig. 2 presents a breakthrough curve for the Pb (II) biosorption onto the immobilized cells of *P. sanguineus* at different bed heights of 5, 9 and 13 cm, respectively. The concentration of the Pb (II) solution was fixed at 100 mg L^{-1} and pH 4 for all the bed heights studied. The

preliminary results showed that pH 4 was an optimum pH for Pb (II) removal. It was fed into the column and maintained at 4 ml min^{-1}. The results showed that the Pb (II) uptake was increased from 11.51 to 19.65 mg g^{-1}, as the bed height was increased from 5 to 13 cm. With the increase of the bed height, more binding sites were available for the biosorption to occur (Vijayaraghavan *et al.*, 2005). The saturation of the immobilized cells of *P. sanguineus* was achieved nearly 38 hours at 5 cm bed height. However, at a higher bed height (13 cm), the saturation was obtained after 144 hours. Malkoc and Nuhoglu (2006) stated that higher bed height would result in broadened mass transfer zone, thus increased the saturation period for the metals onto immobilized cells.

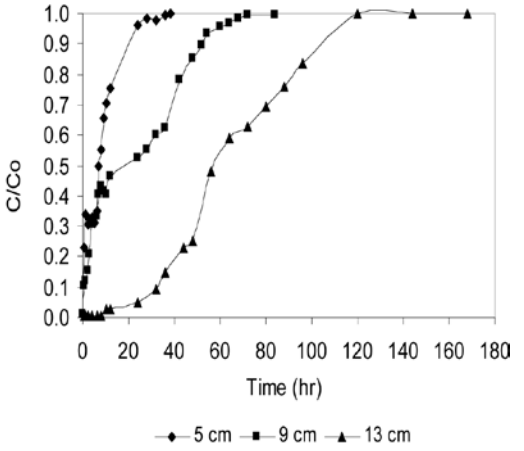

Fig. 2: The breakthrough curves of the Pb (II) biosorption onto immobilized cells of P. sanguineus *at different bed heights (Condition: 100 mg L^{-1} Pb (II), flow rate: 4 ml min^{-1}, pH 4.0)*

The Effect of the Flow Rate

Fig. 3 presents the breakthrough curve of the Pb (II) biosorption in a column at the different flow rates which ranged from 4 to 12 ml min^{-1}. The experiments were carried out at a constant initial Pb (II) concentration (100 mg L^{-1}), pH 4 and 9 cm bed height. The result (*Fig. 3*) revealed that the Pb (II) uptake was decreased with the increase in the flow rate. This was due to the insufficient contact time for the Pb (II) ions to be adsorbed by the immobilized cells of *P. sanguineus* (Ko *et al.*, 2000; Vijayaraghavan *et al.*, 2005). As illustrated in *Fig. 3,* a steeper breakthrough curve was observed at a flow rate of 12 ml min^{-1}, when the breakthrough time decreased. A similar phenomenon was reported on cobalt (II) and nickel (II) biosorption by seaweeds and heavy metals removal in fixed bed column by *P. sanguineus*, respectively (Zulfadhly *et al.*, 2001; Vijayaraghavan *et al.* 2005).

The Effect of the Initial Metal Concentrations

Fig. 4 shows the breakthrough profiles of the Pb (II) biosorption at different initial Pb (II) concentrations with bed height 9 cm, and the flow rate of 4 ml min^{-1} and pH 4. When the initial Pb (II) concentration was increased from 50 to 300 mg L^{-1}, the Pb (II) uptake was also found to increase from 21.74 to 25.73 mg g^{-1}. At a higher initial Pb (II) concentration, the biosorbent was saturated earlier, thus resulted in a faster breakthrough and exhaustion time (Zulfadhly *et al.*, 2001; Malkoc and Nuhoglu, 2006). At a lower concentration of Pb

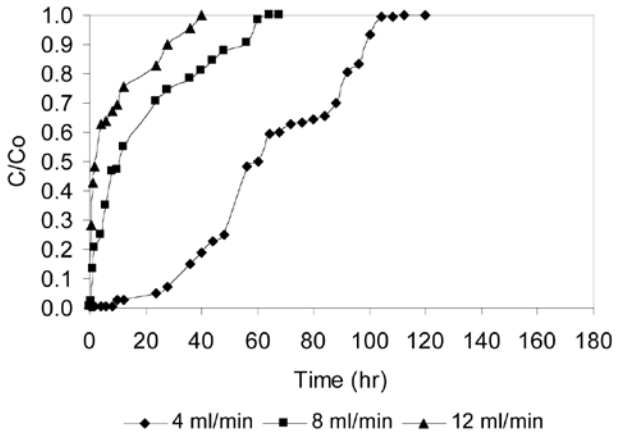

Fig. 3: Breakthrough curves of Pb (II) biosorption onto immobilized cells of P.sanguineus *at different flow rates (Condition: 100 mg L^{-1} Pb (II), bed height= 9 cm, pH 4.0)*

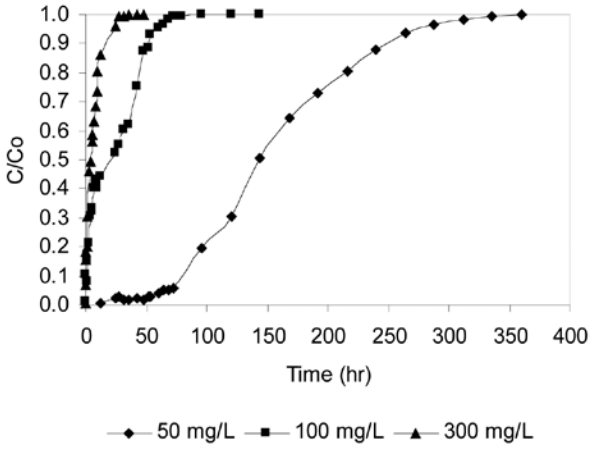

Fig. 4: The breakthrough curves of Pb (II) biosorption onto immobilized cells of P. sanguineus *at different initial Pb (II) concentrations (Condition: 100 mg L^{-1} Pb (II), bed height= 9 cm, pH 4.0)*

(II) solution, less driving force was observed between the metal ions and the immobilized cells of *P. sanguineus,* resulting in a broadened mass transfer zone (Malkoc and Nuhoglu, 2006). The adsorption efficiency, at different bed heights, flow rates and initial metal concentrations, is presented in Table 1.

Regeneration

Regeneration of biosorbent after biosorption process is very important to reduce the process cost in a continuous operation (Vijayaraghavan *et al.*, 2005). *Fig. 5* shows the desorption curve of the Pb (II) ions through a packed bed of *P. sanguineus* by passing 0.1 M HCl as an elution agent. The biosorbents were reused up to two biosorption-desorption cycles. It was observed that the elution efficiency was up to 85% for a complete recovery of Pb (II) ions and more than 20 L of 0.1 M HCl was used. After two biosorption-desorption cycles,

TABLE 1
Adsorption efficiency at different bed heights, flow rates and initial metal concentrations

C_o (mg L^{-1})	*H* (cm)	*F* (ml min^{-1})	Adsorption efficiency (%)
100	5	4	81
100	9	4	74
100	13	4	65
100	9	8	19
100	9	12	15
50	9	4	60
300	9	4	89

a significant biosorbent weight loss was observed, and this suggested that it was no longer suitable to be used in the next cycle. As reported by a few researchers, cells that were exposed to an acidic elutant might face a physical-chemical damage of the biosorbent structure which resulted in both weight loss and reduction of the biosorption capacity in a subsequent cycle (Tuzun *et al.*, 2005; Vijayaraghavan *et al.*, 2005). These can be seen as tabulated in Table 2. The comparison of the Pb (II) biosorption by various biosorbents is shown in Table 3.

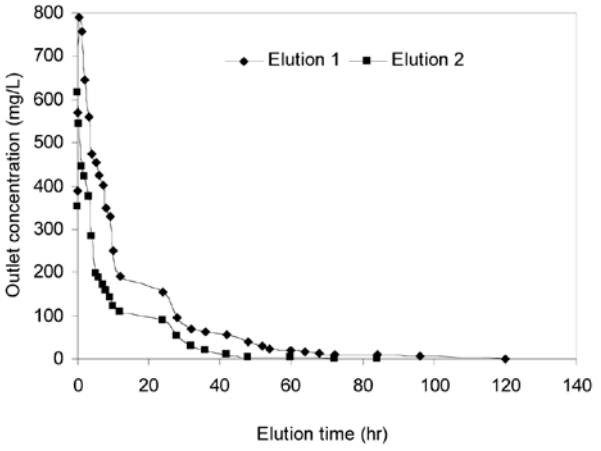

Fig. 5: The breakthrough curves of Pb (II) desorption

TABLE 2
Elution parameters for two biosorption-desorption cycles

Metal	Cycle No	Uptake capacity (mg g^{-1})	Metal removal (%)	Time for Elution (hr)	Elution efficiency (%)
Pb	1	25.7	88.8	120	85
	2	21.0	75.6	84	50

TABLE 3
Comparison of Pb (II) biosorption by various biosorbents

Metal	Biosorbent	Metal Removal (%)	Elution efficiency (%)	Reference
Pb	Immobilized *P. sanguineus*	88.8	85	This study
	Aspergillus niger beads	50	99	Kapoor and Viraraghavan, 1998
	Immobilized bacterial Biomass		98	Chang *et al.* 1998
	Calcium treated Anaerobic biomass	50	80	Hawari and Mulligan, 2006
	Immobilized *Microcytis aeruginosa*	80	80	Jian *et al.* 2005

CONCLUSIONS

The biosorption of the Pb (II) ions was examined using immobilized cells of *P. sanguineus* in a packed bed column, and the following conclusions are therefore summarized: Immobilized cells of *P. sanguineus* cell were found to be capable of removing 88.8 % of Pb (II) ions from aqueous solutions.

The increase in bed height and initial Pb (II) concentration increased the metals uptake in the column. The contact time in the column, at a higher flow rate, resulted in a decrease of the metal uptakes. The column regeneration, using 0.1 M HCl, was carried out for two biosorption-desorption cycles; the results indicated a significant biosorbent weight loss.

ACKNOWLEDGEMENT

The authors acknowledge Universiti Sains Malaysia (Acc No: 6035132) for the financial support of this research.

REFERENCES

Annadurai, G., Lai, Y.L. and Jiunn, F.L. (2007). Biodegradation of phenol by *Pseudomonas pictorium* on immobilized with chitin. *African Journal of Biotech, 6*(3), 296-303.

Arica, M.Y., Arpa, C., Ergene, A., Bayramoglu, G. and Genc, O. (2003). Ca-alginate as a support for Pb (II) and Zn (II) biosorption with immobilized *Phanerochaete chrysosporium. Carbohydrate Polymer, 52*, 167-174.

Arica, M.Y., Kacar, Y. and Genc, O. (2001). Entrapment of white-fungus *Trametes versicolor* in Ca alginate beads: Preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. *Biores. Technol, 80*: 121-129.

Bayramoglu, G., Bektas, S. and Arica, M.Y. (2003). Biosorption of heavy metals on immobilized white-rot fungus *Trametes versicolor. J. Hazard. Mater, B101*, 285-300.

Chang J.S., Huang, J.C., Chang C.C. and Tarn, T. (1998). Removal and recovery of lead fixed-bed biosorption with immobilized bacterial biomass. *Wat. Sci. Technol.*, *38*, 171-178.

Cordero, B., Lodeiro, P., Herrero, R. and Sastre de Vicente, M.E. (2004). Biosorption of cadmium by *Fucus spiralis*. *Environ. Chem.*, *1*, 180-187.

Cruz, C.C.V., Da Costa, A.C.A., Henriques, C.A. and Luna, A.S. (2004). Kinetic modelling and equilibrium studies during cadmium biosorption by dead *Sargassum* sp. biomass. *Biores. Technol.*, *91*, 249-257.

Hawari, A.H. and Mulligan, C.N. (2006). Heavy metals uptake mechanisms in a fixed-bed column by calcium-treated anaerobic biomass. *Process. Biochem.*, *41*, 187-198.

Jian, Z.C., Xian, C.T., Jun, X., Tao, Z. and Zhi, L.L. (2005). Biosorption of lead, cadmium and mercury by immobilized *Microcystis aeruginosa* in a column. *Process. Biochem.*, *40*, 3785-3679.

Jianlong, W., Horan, N., Stentiford, E. and Yi, Q. (2000). The radial distribution and bioactivity of *Pseudomonas* sp immobilized in calcium alginate gel beads. *Process. Biochem.*, *35*, 465-469.

Kapoor, A. and Viraraghavan, T. (1997). Heavy metal biosorption sites in *Aspergillus niger*. *Biores. Technol.*, *61*, 221-227.

Kapoor, A. and Viraraghavan, T. (1998). Removal of heavy metals from aqueous solution using immobilized fungal biomass in continuous mode. *Wat. Res*, *32*, 1968-1977.

Kim, S.K., Park, C.B., Koo, Y.M. and Yun, H.S. (2003). Biosorption of cadmium and copper ions by *Tricoderma reesei* RUT C30. *J. Ind. Eng. Chem.*, *9*, 403-406.

Ko, D.C.K., Porter, J.F. and McKay, G. (2000). Optimised correlations for the fixed-bed adsorption of metal ions on bone char. *Chem. Eng. Sci.*, *55*, 5819-5829.

Malkoc, E. and Nuhoglu, Y. (2006). Removal of Ni (II) ions from aqueous solutions using waste of tea factory: Adsorption on a fixed-bed column. *J. Hazard. Mater.*, *B135*, 328-336.

Padmesh, T.V.N., Vijayaraghavan, K., Sekaran, G. and Velan, M. (2005). Batch and column studies on biosorption of acid dyes on fresh water macro alga *Azolla filiculoides*. *J. Hazard Mater, B125*, 121-129.

Say, R., Denizli, A. and Arica, M.Y. (2001). Biosorption of cadmium (II), lead (II) and copper (II) with the filamentous fungus *Phanerochaete chrysosporium*. *Biosres. Technol.*, *76*, 67-70.

Ting, Y.P. and Sun, G. (2000). Use of polyvinyl alcohol as a xall entrapment matrix for copper biosorption by yeast cells. *J. Chem. Technol. Biotechnol.*, *75*, 541-546.

Tuzun, I., Bayramoglu, G., Yalcin, E., Basaran, G., Celik, G. and Arica, Y.M. (2005). Equilibrium and kinetic studies on biosorption of Hg (II), Cd (II) and Pb (II) ions onto microalgae *Chlamydomonas reinhardtii*. *J. Environ. Manage. 77*, 1-8.

Vijayaraghavan, K., Jegan, K., Palanivelu, K. & Velan, M. (2005). Biosorption of copper, cobalt and nickel by marine green alga *Ulva reticulate* in a packed column. *Chemosphere.*, *60*, 419-426.

Volesky, B., Weber, J. and Park, J.M. (2003). Continuous-flow metal biosorption in a regenerable *Sargassum* column. *Wat. Res*, *37*, 297-306.

Yan, G. and Viraraghavan, T. (2001). Heavy metal removal in a biosorption column by immobilized *M. rouxii* biomass. B*iores. Technol.*, *78*, 243-249.

Yan, G. and Viraraghavan, T. (2003). Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. *Wat. Res*, *37*, 4486-4496.

Zulfadhly, Z., Mashitah, M.D. and Bhatia, S. (2001). Heavy metals removal in fixed-bed column by the macro fungus *Pycnoporus sanguineus*. *Environ. Pollut.*, *112*, 463-470.