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UTILIZATION OF CYANOBACTERIUM *PHORMIDIUM* SP., TO PRODUCE IMMOBILIZED HYBRID DISC BIOSORBENT FOR THE REMOVAL OF CD^{2+} FROM AQUEOUS SOLUTION

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Abstract

An indigenous strain of blue green microalga was entrapped within reticulated fibrous network of loofa sponge disc to produce immobilized hybrid disc biosorbent (IHDB) and successfully used for the removal and recovery of Cd^{2+} from aqueous solution. The two biosorbents used as the symbiotic building block to produce IHDB, were the filamentous blue green alga *Phormidium* sp. (B1) and loofa sponge discs (B2). Maximum biosorption capacity of B1 and B2 was noted respectively to be 37.06 and 5.32 mg Cd^{2+} g^{-1} biosorbent. However, when the two biosorbents were combined to form IHDB, the biosorption capacity (48.53 mg g^{-1}) was increased by 30.95%, 812.21%, respectively as compared to the ability of B1 and B2 when used alone, and by14.51% than the sum of individual abilities of the two biosorbents. The kinetics of Cd^{2+} removal by IHDB was rapid, with 91.81% of Cd^{2+} biosorption occurring within first 30 min., and equilibrium was reached after 60 min of contact. The Langmuir and Freundlich adsorption isotherm models were used for mathematical description of the sorption equilibrium. Equilibrium data fitted very well to the Langmuir model in the studied range of concentration (5-200 mg l^{-1}). The biosorbed Cd^{2+} was desorbed by washing the IHDB with dilute HCl (50 mM) and regenerated IHDB was reused in seven biosorption-desorption cycles without an apparent decrease in metal biosorption capacity. The metal removing capacity of IHDB was also tested in continuous flow fixed-bed column bioreactor and found to be highly effective in removing Cd^{2+} from aqueous solution. The results suggested that IHDB could be used as low-cost and environment-friendly biosorbent for the efficient removal of Cd^{2+} from aqueous solution.

Introduction

With the development of industrialization and human activities, the discharge of waste and wastewater containing heavy metals to environment has increased. Mine draining, metal industries, petroleum refining, tanning, photographing processing and electroplating are some of the main sources of heavy metals (Volesky, 2001). Lead, cadmium, nickel, chromium, copper and zinc are the heavy metals found in such wastewater discharges. Environmental contamination by these heavy metals is a serious problem due to their incremental accumulation in the food chain (Volesky & Schiewer, 1999). Unlike most organic wastes and the microbial load in aquatic bodies, metal contaminants are not biodegradable, tending to accumulate in living organisms, thus becoming a permanent burden on ecosystems (Bailey *et al*., 1999). Industrial effluents, particularly those containing heavy metals, are thus a cause of serious hazard to human health and other forms of life. Increasing attention is, therefore, being paid to the development of know-how for their removal from metal bearing effluents before their discharge into water bodies and natural streams. Among these heavy metals, Cd^{+2} is the most toxic metal and is known to cause renal dysfunction, bone degeneration, lung insufficiency, liver damage and hypertension in humans (Nordberg *et al*., 1993). On the basis of these adverse health effects, Cd^{+2} has been included in the red list of priority pollutants by the Department of Environment, UK (Anon., 1991) and in the black list of EEC dangerous substances directive (Anon., 1976). US Environment Protection Agency has also classified Cd^{2+} as Group B1 carcinogen (Anon., 1999). The most commonly used procedures for the treatment of Cd^{+2} containing effluents include chemical precipitation, evaporation, ion exchange, and membrane separation. Techno-economic considerations, however, limit their wide-scale applications (Atkinson, 1998). Therefore, the need for the development of economical, effective and safe methods for the removal of Cd^{+2} has led to the search for alternative procedures.

The use of biological materials in general, and microalgae including cyanobacteria in particular, has received considerable attention during recent decades for the removal of heavy metals as the environment friendly alternative technology (Romera *et al*., 2006). Microalgae, because of their large surface area and high binding affinity, have been reported to effectively remove heavy metals from wastewaters (Roy *et al*., 1993). The application of microalgal biomass as a biosorbent on a commercial scale, however, has been hindered by operational limitations associated with their physical characteristics, such as small particles with low density, poor mechanical strength and low rigidity, and solid-liquid separation (McHale & McHale, 1994). As an alternative, a number of agro-based plant waste materials, such as coconut fibres (Espinola *et al*., 1999), black gram husk (Saeed & Iqbal, 2003), fibrous network of papaya wood (Saeed *et al*., 2005), petiolar felt-sheath of palm (Iqbal & Saeed, 2002) and mungbean husk (Saeed *et al*., 2009) have been tested as low-cost metal biosorbents. The adsorption capacity of these agro-based plant waste materials is, however, generally low which in practical terms means their use in large volumes, rendering their application impractical. To overcome the problems associated with the application of both microbial and agro-based materials as the biosorbent, a novel idea of producing an immobilized hybrid disc biosorbent (IHDB) was considered and an innovative IHDB was produced by combining two previously known biosorbents, namely, the filamentous biomass of *Phormidium* sp. (B1) and the fibrous network of loofa sponge (B2) using a simple technique of immobilization (Akhtar *et al*., 2003) in which two known metal sorbents act as complementing partners. Application of this IHDB is reported here as an innovative, inexpensive and environment-friendly biosorbent, for the first time, for the removal of Cd^{+2} from aqueous solution. Attempts were also made to characterize the various biosorption process parameters such as pH, equilibrium time, initial metal ion and biosorbent concentrations and adsorption isotherms modelling influencing the metal adsorption–desorption in anticipation of the potential use of this newly developed immobilized biosorption system to large scale metal recovery systems in near future.

Materials and Methods

Organisms and culture medium: An indigenous strain of *Phormidium* sp., isolated from wastewater bodies containing effluents from electroplating, leather and textile industries was used in this study. The culture was maintained in test tube slants of Bold's basal agar medium (Nichols & Bold, 1965) at 25±2 ^{o}C under continuous illumination with cool-white

fluorescent light at the intensity of 50 µE m^{-2} s^{-1}. Biomass for inoculum and metal sorption was prepared by growing axenic algal cultures to exponential phase of growth in 100 ml Bold's medium contained in 250 ml Erlenmeyer flasks shaken at 100 rpm and maintained at 25±2 °C under continuous illumination with cool-white light at the same intensity as for algal culture maintenance. The cyanobacterial biomass was harvested, washed with deionized water, and freeze dried for metal biosorption studies.

Preparation of IHDB: Loofa sponge, used as an immobilizing matrix for the entrapment of cyanobacterial biomass, was obtained from the dry fruit of Loofa (*Luffa cylindrica* (Linn.) Roem , member of the family Cucurbitaceae. The matured dried fruit of loofa is cylindrical to fusiform in shape (Fig. 1a), 25 to 60 cm long, 5 to 10 cm in diameter. The sponge is recovered from the dried fruit by retting the exocarp and other fleshy tissues in water (Fig. 1b). The sponge is a cushion like structure made up of well organized fibrous network (Fig. 1c-d). The fibres, 150-800 µm in dia., are interconnected forming a multilayered network having 2-4 mm mesh opening overlapping each other (Fig. 1e). The sponge was cut into discs of approximately 22.5 mm dia, 2–3 mm thick, soaked in boiling water for 30 min, thoroughly washed under running tap water, and left for 24 h in distilled water, changed 3–4 times. The sponge discs were then oven dried at 70°C till constant weight. For entrapment of *Phormidium* cells, four loofa sponge discs (B2) were accurately weighed, transferred to 100 ml of Bold's medium in 250 ml Erlenmeyer flasks and autoclaved for 20 min at 120 °C at 1.06 kg cm^{-2} pressure. Each of these flasks was inoculated with 5 ml of exponential phase *Phormidium* cells (B1) and incubated on a rotary shaker at 100 rpm under continuous cool-white light at an intensity of 50 µE m^{-2} s^{-1} for 2 weeks. The sponge discs with the entrapped algal mass in the culture flasks, were washed thoroughly with fresh algal culture medium to remove any free algal cells, transferred to 100 ml fresh algal culture medium, and incubated under the same set of initial culture conditions. After three weeks of incubation, *Phormidium* biomass (B1) was found entrapped within the loofa sponge (B2) to form an immobilized hybrid disc biosorbent (IHDB) (Fig. 2a). The IHDB so produced was harvested at the day 24 of incubation (Fig. 2b), washed twice with distilled water and stored at 4 °C until use. Amount of cyanobacterial biomass entrapped within the sponge disc was determined as the difference between constant dry weights of the sponge discs with and without the immobilized cyanobacterial mass.

Biosorption studies: The biosorption of Cd^{2+} by IHDB matrix from aqueous solution was carried out in batch biosorption-equilibrium studies. Desired concentrations of Cd^{2+} solutions were prepared by diluting 1,000±2 mg l^{-1} standard Cd^{2+} stock solution ($Cd(NO_3)_2$, Merck Ltd., Poole, UK). pH of the solution was adjusted to 5.0, using 0.1 M NaOH. Fresh dilutions were used for each biosorption study. The biosorption capacity of B1, B2 and IHDB (100 mg) was determined by contacting 100 ml Cd^{2+} solutions of known concentrations (10-500 mg l^{-1}) in 250 ml Erlenmeyer flasks. The Cd^{2+} solution, incubated with the IHDB, was shaken on an orbital shaker at 100 rpm in tightly stoppersed flasks at 25±2°C. B1 was removed from metal solution by centrifugation at 5,000 rpm for 5 min, whereas B2 and IHDB were separated from the solution by simple decantation. Residual concentration of Cd^{2+} in the metal supernatant solutions was determined using atomic absorption spectrophotometer (UNICAM-969, Unicam Cambridge, UK). Desorption of Cd^{2+} was performed by 50 mM HCl solution. IHDB loaded with Cd^{2+} ions were contacted with desorption medium at room temperature and agitated on orbital shaker at 100 rpm for 60 min. The biosorbent was removed and the supernatant was analysed for Cd^{2+} released into the solution by atomic absorption spectrophotometer.

Biosorption-desorption cycles: In order to determine the reusability of the IHDB, adsorption-desorption cycles were repeated seven times by using the same IHDB. Desorption of Cd^{2+} was done using 50 mM HCl solution. The IHDB loaded with Cd^{2+} ions was contacted with desorption medium at room temperature and agitated on orbital shaker at 100 rpm for 60 min. The biosorbent was removed and the supernatant was analysed for Cd^{2+}, released into the solution, by atomic absorption spectrophotometer.

Continuous removal of Cd^{2+} by IHDB packed in fixed bed column bioreactor: To demonstrate the biosorption potential of IHDB in a continuous flow system, IHDB (2.04±0.11 g of *Phormidium* biomass entrapped on loofa sponge) was packed in an up-flow fixed bed column bioreactor (2.7 cm in diameter and 30 cm in height, packing height 25 cm). Cd^{2+} solution (10 mg l^{-1}, pH 5.0) was then pumped upwards through the column at a flow rate of 5 ml min^{-1}. Samples were collected at regular intervals from the effluent to measure residual Cd^{2+} concentrations. As the bed was saturated, the Cd^{2+} loading was terminated, and the bed was eluted with 50 mM HCl solution to recover the loaded Cd^{2+} ions. The regenerated bed was washed thoroughly with deionized water before use in the next adsorption cycle.

Results and Discussion

IHDB preparation: Filamentous biomass of cyanobacterium *Phormidium* sp., (B1) was noted to be fully entrapped within the fibrous network of loofa sponge disc (B2) in about 21 days to form IHDB (Fig. 2b). The IHDB, so obtained, was harvested at the end of the 24th day. Microscopic observations of the IHDB indicated a uniform growth of B1 on the fibres of loofa sponge discs (B2) indicating that filaments of blue green alga *Phormidium* sp., are not localized in isolated patches but are intra-woven into a continuous mass.

For the production of loofa sponge immobilized disc biosorbent, 5 ml of three weeks old stationary phase culture of *Phormidium* sp., was added to 250 ml Erlenmeyer flasks containing 100 ml Bold's medium and four discs of loofa sponge. The flasks were incubated at 25°C on orbital shaker, shaken at 100 rpm, under continuous illumination with cool white light at an intensity of 50 µE m^{-2} s^{-1}. Flasks with no loofa sponge discs were used as control for the growth of free biomass of *Phormidium* sp. Both visual and microscopic examination of loofa sponge discs revealed *Phormidium* sp., immobilization on the sponge fibres in 5 to 7 days of incubation. The sponge pieces, nevertheless, were continued to be incubated in the culture medium for further 3 days to allow complete and stable immobilization. The immobilized cyanobacterial biomass was, thereafter, subcultured in fresh culture medium and maintained in batch culture for 24 days. The fibrous network of the sponge was noted to be covered by immobilized cyanobacterial filaments during this period (Fig. 2b).

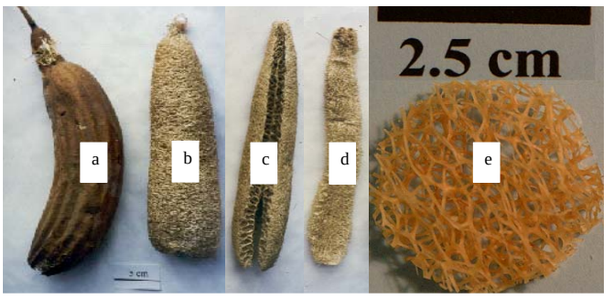


Fig. 1. (a) Matured dried fruit of *Luffa cylindrica*, (b) Loofa sponge recovered from (a) after removing the hard cover of the fruit, (c-d) longitudinally cut pieces of loofa sponge, (e) sponge discs(2.5 cm in diameter) showing multilayered fibrous network.

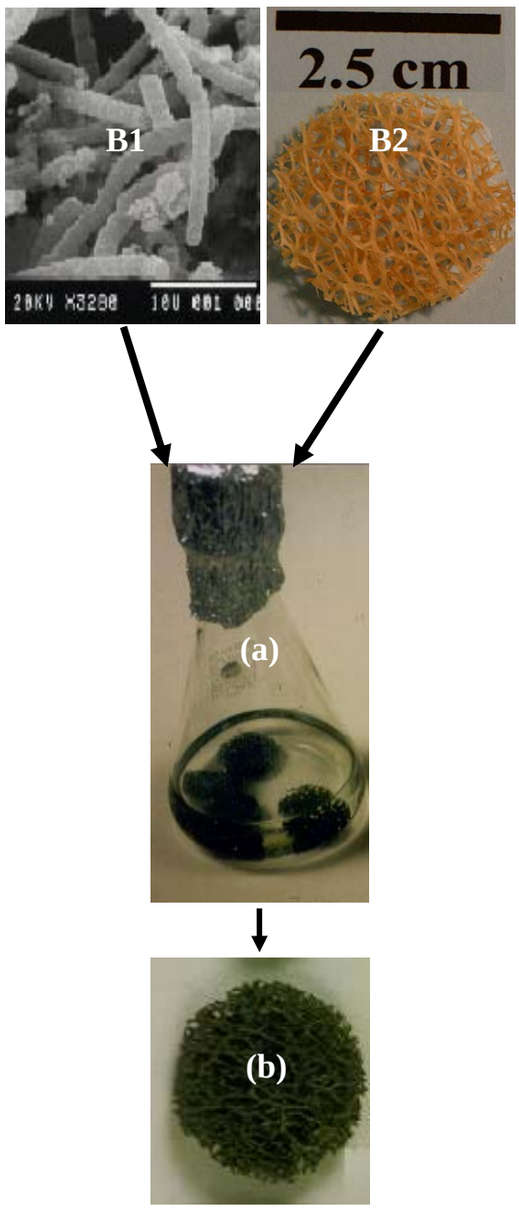


Fig. 2. Filamentous biomass of *Phormidium* sp. (B1), loofa sponge disc (B2) and immobilized hybrid disc biosorbent (a) IHDB produced by entrapment of *Phormidium* sp. within loofa sponge discs, (b) harvested IHDB at the end of 24th day.

Biosorption performance of IHDB: To demonstrate the metal binding capacity of IHDB as a new biosorption system, IHDB was contacted with Cd^{2+} solution (100 mg l^{-1}, pH 5.0) in batch experiments. Fig. 3 shows the biosorption capacity of IHDB as a function of time. The biosorption capacity of B1 and B2 has also been included in Fig. 3 for comparative purpose. Rapid Cd^{2+} biosorption rates were observed during the first 30 min of contact with IHDB; 91.81% of the total metal was adsorbed by the IHDB, compared with 79.62% by B1 and 60.73% by B2 individually. Biosorption equilibrium, however, was achieved by all the three biosorbents at about 60 min with the maximum uptake of 35.26, 4.93 and 44.73 mg Cd^{2+} g^{-1} for B1, B2 and IHDB, respectively, from 100 mg l^{-1} Cd^{2+} solution. This rapid rate of metal sorption by IHDB has significant advantage over the other microbial immobilized biosorbents reported previously. For example, yeast cells immobilized in polyvinyl alcohol and alginate removed copper very slowly, achieving equilibrium in 12 and 24 h, respectively, which was about 24 and 48 times higher than the time taken by free biomass (30 min) in the same study (Ting & Sun, 2000). Similarly, microbial biomass, from activated sludge, immobilized in sodium alginate beads, took 15 h to achieve equilibrium for the removal of Cd^{2+}, which was 100 times higher than the time taken by the free cells (Gourdon *et al*., 1990). These authors suggested that the observed slow rate of Cd^{2+} uptake by alginate beads was limited by diffusion of Cd^{2+} through the gel matrix. A slower rate of metal uptake by the hydrophilic polyurethane foam-immobilized biomass of *Ascophyllum nodosum* was also noted by Alhakawasti & Banks (2004), who reported that the foam-immobilized biomass took about 320 min to reach equilibrium, in comparison with free biomass where equilibrium for metal uptake was reached in less than 90 min. These authors suggest that the slower adsorption rate of the immobilized biomass may be attributed to the restriction encountered by the solute to diffuse through the foam membrane for reaching the functional groups on the biomass surface. In the case of IHDB, no such problem was presented due to the surface immobilization of fungal hyphae on the highly porous matrix of papaya wood and thus the process of biosorption was completed within 60 min.

Metal removal capacity of IHDB: Maximum metal sorption capacity of IHDB was investigated by contacting the biosorbent with varying concentrations (5-200 mg l^{-1}) of Cd^{2+}. Increase in the Cd^{2+} uptake was noted with an increase in metal ions concentration in the solution until it reached the maximum capacity of 48.53 mg Cd^{2+} g^{-1} biosorbent (Fig. 4). This maximum Cd^{2+} removal capacity of IHDB was observed to be 30.94% and 15.32% higher, as compared to the ability of fungal biomass (B1) when used alone, and the sum of separate individual abilities of biosorbents B1 and B2, respectively. The removal of Cd^{2+} by B2 was found to be 5.32 mg g^{-1}. Though it was not possible to predict how much of it contributed to the 48.53 mg g^{-1} Cd^{2+} biosorbed by IHDB, yet most of it is likely to have been adsorbed on the expanded surface area of this unique biosorbent provided by the cynobacterium biomass (B1) immobilized along the outer surface of the fibres of B2. From these results, nevertheless, it is clear that the use of B2 as an immobilization matrix for B1 has significantly enhanced the biosorption capacity of the IHDB and has caused no negative effect on the biosorption process. This is a significant achievement in the field of environment biotechnology as most of the previously reported microbial immobilized biosorbents are known to have resulted in a significant decrease in the metal uptake in comparison with free cell biomass used as biosorbents. For example, a 27.66% and 62.63% reduction in the sorption of Cd^{2+} was noted when cells of *Spirulina platensis*

were immobilized, respectively, in alginate and silica gel beads as compared to free cells (Rangsayatorna *et al*., 2004), while Lopez *et al*., (2002) also noted a 60% decrease in the rate of metal sorption by *Pseudomonas fluorescens* cells when immobilized in agar beads, as compared with free cells. These reductions have been projected to be due to limitations in the movement of metal ions, or the masking of active sites on the biosorbent (Praksham *et al*., 1999). Moreover, part(s) of the cell surface might be shielded by the gel matrix and would thus not be available for metal binding (Rangsayatorna *et al*., 2004). In the present study, surface immobilization of *Phormidium* sp. (B1) on the fibrous network of loofa sponge (B2) provides a direct contact of biomass to metal solution which is well suited for biosorption than the enclosed or beaded immobilization systems based on polymeric gel structures.

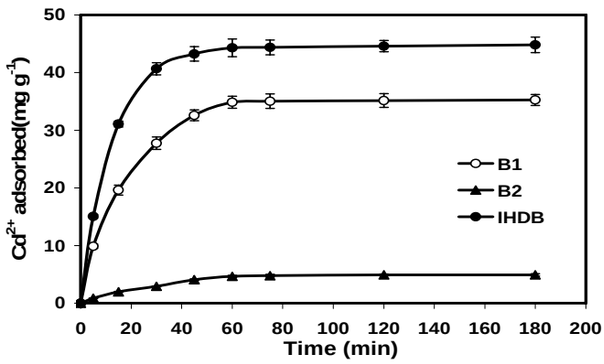


Fig. 3. Biosorption of Cd^{2+} from 100 mg l^{-1} metal solution, pH 5, by 1 g l^{-1} of *Phormidium* sp. (B1), loofa sponge (B2) and immobilized hybrid disc biosorbent (IHDB) as related to the time of contact during orbital shaking at 100 rpm at 25°C.

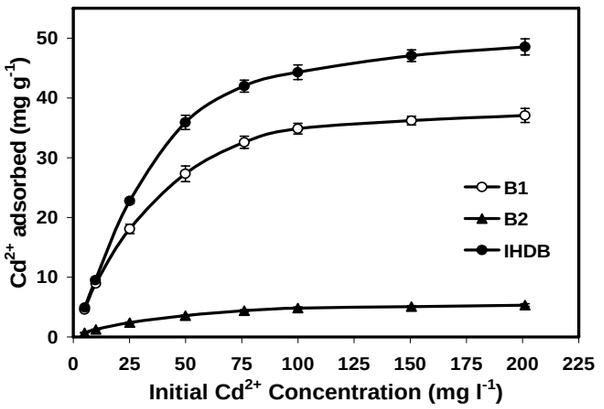


Fig. 4. Effect of initial metal ion concentration (on biosorption of Cd^{2+} from aqueous solution by *Phormidium* biomass (B1), loofa sponge disc (B2) and immobilized hybrid disc biosorbent (IHDB): 100 ml of Cd^{2+} solution (5-200 mg l^{-1}, pH 5) was mixed with each biosorbent (1 g l^{-1}) in shake flask at 10 rpm and 25 °C for 60 min.

Adsorption isotherms: In order to establish if the IHDB could be modelled using adsorption isotherms, the two most commonly used isotherm equations, Langmuir and Freundlich, were used. The Langmuir isotherms assumes monolayer adsorption and is given by the following equation (linear form):

$$\frac{C_e}{q} = \frac{1}{bq_{\max}} + \frac{C_e}{q_{\max}} \qquad \text{(Eq. 1)}$$

where q and q_{max} are the observed and maximum uptake capacities (mg g^{-1} biosorbent); C_e is the equilibrium concentration (mg l^{-1} solution); *b* is the equilibrium constant (l mg^{-1}).

The Freundlich isotherm is an empirical equation based on sorption at a heterogeneous surface and can be presented as:

$$\log q_{eq} = \log K_F + [(1/n)(\log C_{eq})] \qquad \text{(Eq. 2)}$$

where K_F and *n* are the Freundlich constants characteristic of the system. K_F and n are indicators of adsorption capacity and adsorption intensity, respectively. From linearized Langmuir and Freundlich isotherms of Cd^{2+} (Fig. 5a & b), Langmuir and Freundlich constants and their correlation coefficients (r^2) were calculated (Table 1). As seen from the Table, very high regression correlation coefficient (>0.991) was found for Langmuir isotherms than the Freundlich isotherms model, which suggests that the adsorption process by IHDB was better defined by Langmuir than by the Freundlich.

Continuous removal of Cd^{2+} by IHDB in fixed bed column bioreactor: To examine the biosorption potential of IHDB in continuous flow system, IHDB was packed in an up-flow fixed bed column bioreactor. A Cd^{2+} solution of 10 mg l^{-1}, pH 5.0, was pumped through a column at a flow rate of 5 ml min^{-1}. The breakthrough curve obtained is presented in Fig. 6a. The Cd^{2+} loading curve showed an excellent, clear zone (i.e. 100% removal) before the breakthrough point. Approximately 4.5 l of 10 mg l^{-1} Cd^{2+} solution was treated completely before breakthrough occurred. In the loading stage, a total of 83.1±2.73 mg of Cd^{2+} was accumulated in the column. The number of 83.1 mg Cd^{2+} was obtained by numerical integration of the whole breakthrough curve. Thus, the Cd^{2+} biosorption capacity of the IHDB in the column operation was 49.47 mg of Cd^{2+} g^{-1} biosorbent. Desorption of Cd^{2+} sorbed on the IHDB in the fixed bed column was carried out with 50 mM HCl. The desorption equilibrium was achieved on the passage of 500 ml HCl (Fig. 6b). The metal desorbed during the passage of this volume was 82.7 mg, which was 99.52% of the 83.1 mg total metal biosorbed in the fixed bed.

Reusability of IHDB in repeated adsorption-desorption cycles: In order to assess the reusability of the IHDB, a series of adsorption-desorption experiments were performed. The IHDB undergoing successive adsorption-desorption cycles retained good metal adsorption capacity even after seven cycles (Fig. 7). The total decrease in the sorption efficiency of IHDB after seven cycles was only about 5.02%, which shows that IHDB has good potential to adsorb metal ions from aqueous solution and can be used repeatedly. Furthermore, no significant leakage of entrapped biomass or physical breakage of IHDB was observed during seven repeated adsorption-desorption cycles as was noted with other polymeric matrices used in immobilized systems (Rangsayatorna *et al*., 2004; Hu & Reeves, 1997), which ultimately resulted in the loss of biosorption capacity of these immobilized systems.

Conclusions

Efficient Cd^{2+} removing capacity of IHDB observed during the present study and simplicity of the immobilization technique, used to entrap *Phormidium* sp. (B1) on to the loofa sponge fibrous network (B2) to produce the IHDB, indicate the of potential application of this novel and reusable metal biosorbent, which could be used as a significant tool for the development of a low-cost biomaterial-based polishing treatment of heavy metal wastes in industrial effluents.

Table 1. Isotherms model constants and correlation coefficients for biosorption of Cd^{2+} ions from aqueous solution.

Biosorbents	Langmuir isotherms model			Freundlich isotherms model		
	q_{max} (mg g^{-1})	b (l mg^{-1})	r^2	K_F	n	r^2
B1	36.41	0.343	0.992	8.01	2.92	0.952
B2	4.89	0.064	0.996	1.75	2.12	0.961
IHDB	48.13	0.806	0.991	12.54	3.17	0.934

q_{max} is maximum Cd^{2+} uptake (mg g^{-1} biosorbent) and b is the equilibrium constant for Langmuir isotherm model, K_F and n are the Freundlich constants, r^2 is correlation coefficient.

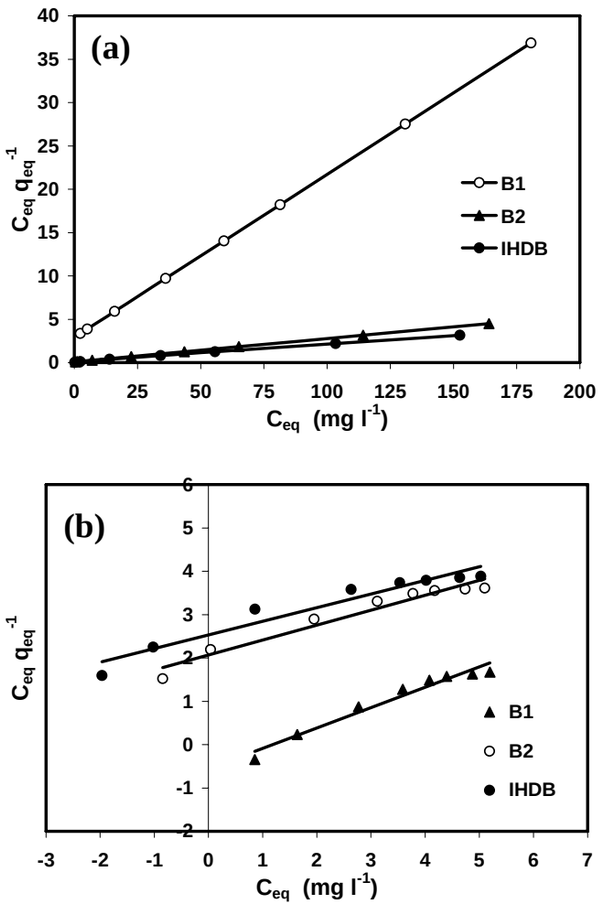
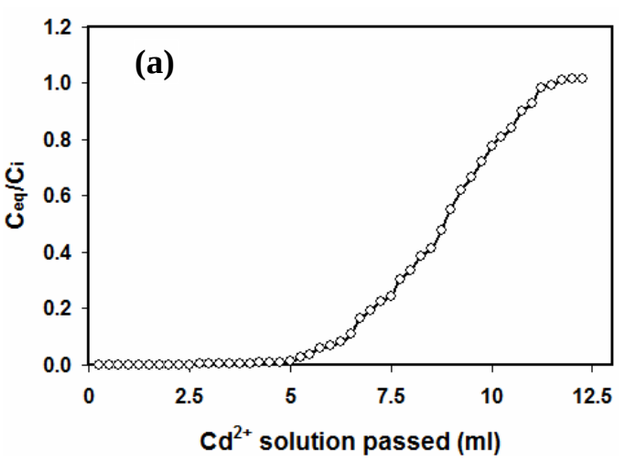


Fig. 5. The linearized (a) Langmuir and (b) Freundlich adsorption isotherms for the sorption of Cd^{+2} by *Phormidium* sp. biomass (B1), loofa sponge (B2) and immobilized hybrid disc biosorbent (IHDB).



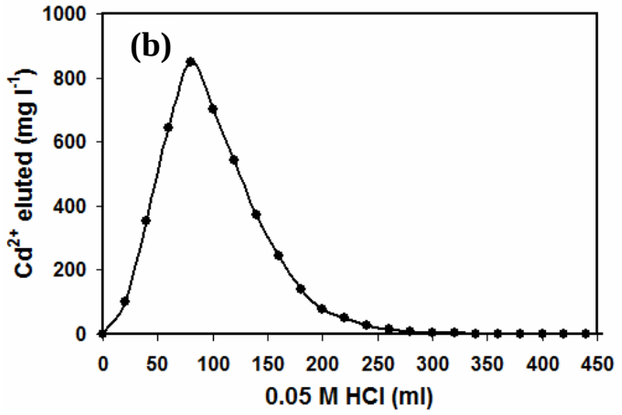


Fig. 6. Biosorption (a) and desorption (b) breakthrough curve for the removal of Cd^{2+} at 10 mg l^{-1} concentration by immobilized hybrid disc biosorbent (IHDB) in a fixed bed column bioreactor.

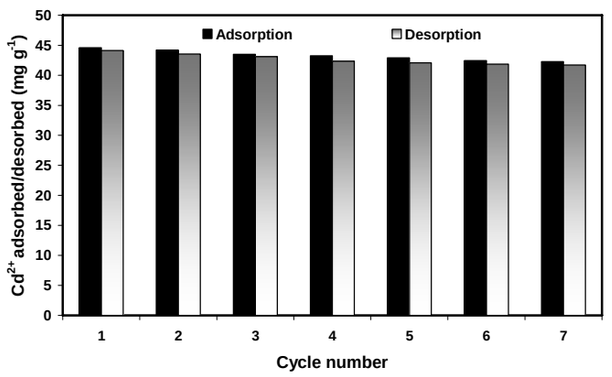


Fig. 7. Biosorption-desorption of Cd^{2+} by immobilized hybrid disc biosorbent (IHDB) in seven consecutive cycles using 50 mM HCl as desorbing agent.

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