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Removal and recovery of nickel(II) from aqueous solution by loofa sponge-immobilized biomass of *Chlorella sorokiniana*: characterization studies

N. Akhtar [a], J. Iqbal [b], M. Iqbal [c,*]

[a] *Department of Biology, Government Islamia College for Women, Cooper Road, Lahore, Pakistan*
[b] *Department of Botany, University of Punjab, Lahore, Pakistan*
[c] *Environment Biotechnology Group, Biotechnology and Food Research Center, PCSIR Laboratories Complex, Lahore, Pakistan*

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Abstract

The biosorption process for the removal of nickel(II) by loofa sponge-immobilized biomass of *Chlorella sorokiniana* (LIBCS), a newly developed immobilized biosorbent, was characterized. Effects of environmental factors on metal uptake capacity of LIBCS were studied and compared with free biomass of *C. sorokiniana* (FBCS). Nickel(II) removal by LIBCS was found to be influenced by pH of the solution, initial metal concentration, and biomass concentration. The biosorption of nickel(II) ions by both LIBCS and FBCS increased as the initial concentration of nickel(II) ions increased in the medium. No loss to biosorption capacity of LIBCS for nickel(II) was found due to the presence of loofa sponge, indeed as compared to FBCS an increase of 25.3% was noted in the biosorption capacity of LIBCS. Maximum biosorption capacities for FBCS and LIBCS were found as 48.08 and 60.38 mg nickel(II)/g, respectively, whereas the amount of nickel(II) ions adsorbed on the plain loofa sponge was 6.1 mg/g. During these biosorption studies, LIBCS exhibited excellent physical and chemical stability without any significant release/loss of microalgal biomass from loofa sponge matrix. The kinetics of nickel(II) removal was extremely fast reaching at equilibrium in about 15 min for LIBCS and 20 min for FBCS. The biosorption equilibrium was well described by the Langmuir and Freundlich adsorption isotherms. The biosorption capacities were found to be solution pH dependent and the maximum adsorption was found at a solution pH 4–5. The LIBCS could be regenerated using 75 mM HCl, with up to 98% recovery. The LIBCS were shown to be robust and stable with little decrease in the nickel(II) uptake capacity when used in consecutive seven biosorption–desorption cycles. Continuous removal of nickel(II) from electroplating effluent by LIBCS packed in fixed bed column bioreactor confirm the possibility of developing a biological treatment process for the removal of toxic metals from authentic wastewater.
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1. Introduction

Wastewater discharge from electroplating, electronics, and metal cleaning industries often contains high concentrations of nickel(II) ions and causes serious water pollutions, unless removed from industrial effluents before discharge. The ingestion of nickel(II) beyond the permissible levels causes various types of acute and chronic disorder in man, such as severe damage to lungs and kidney, gastrointestinal distress (e.g. nausea, vomiting, diarrhea), pulmonary fibrosis and renal edema, and skin dermatitis [1,2]. Traditional physico-chemical methods such as ion exchange and precipitation are often ineffective and/or expensive, particularly when used for the removal of heavy metals at low concentrations (<100 mg/l) [3]. Most of these, furthermore, are based on physical displacement or chemical replacement, generating yet another problem in the form of toxic sludge [4,5], the disposal of which adds further burden on the techno-economic feasibility of the treatment process. Efficient and environment-friendly technologies are, thus, needed to be developed to reduce heavy metal content in wastewaters at discharge to acceptable level at affordable cost.

The search for alternative and innovative wastewater treatment techniques has focussed attention on the use of

* Corresponding author. Fax: +92-42-9230705.
E-mail address: iqbalmdr@brain.net.pk (M. Iqbal).

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biological materials such as algae, fungi, yeast, and bacteria for the metal removal and recovery technologies and has gained importance during recent years [6,7]. Commercial application of these microbial biomass as a biosorbent, however, has been hindered by problems associated with physical characteristics of these materials such as small particle size with low density, poor mechanical strength and rigidity, and solid–liquid separation [8,9]. Immobilization of the biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity, and porous characteristics to the biological material [10]. Among the existing methods for immobilizing the potential microorganisms, entrapment in a polymeric matrix was the most common method. However, immobilization matrices based on polymeric metabolites result in restricted diffusion due to closed embedding structures with low mechanical strength. These difficulties can be overcome by immobilizing the microbial biomass within the loofa sponge. Loofa sponge is a highly porous and strong biomatrix, made of an open network of fibrous support from dry fruit of *Luffa cylindrica*. While loofa sponge has been previously suggested as an immobilization matrix for algal, fungal, and yeast cells [11–13], the use of loofa sponge-immobilized biomass for metal biosorption has not been investigated. Thus, the objective of this study is to investigate the use of loofa immobilized biomass as an alternative low cost biosorbent system for the removal of heavy metals from aqueous solution.

Preliminary studies on this new biosorption system had shown that loofa sponge-immobilized biomass of *Chlorella sorokiniana* (LIBCS) could adsorb heavy metal efficiently [14]. In the present study, attempts were made to characterize the various biosorption process parameters (i.e. pH, equilibrium time, initial metal ion and biosorbent concentration, and adsorption isotherm modeling) 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. Physical and chemical stability and durability of LIBCS during repeated and variable biosorption operational conditions were considered. Furthermore, metal removal potential of LIBCS from authentic wastewater (electroplating effluent) was investigated. Finally, a comparison between biosorption efficiency of free biomass of *C. sorokiniana* (FBCS) and LIBCS was made to highlight the importance of this newly developed immobilized system.

2. Materials and methods

2.1. Organism and culture medium

An indigenous strain of unicellular green microalga *C. sorokiniana* isolated from a wastewater body containing effluents from electroplating and leather industries was used in this study. Biomass for inoculum was grown to stationary phase in 100 ml Bold's medium contained in 250 ml Erlenmeyer flasks, shaken in an orbital shaker at 100 rpm at $25 \pm 2\,^{\circ}$C under continuous illumination with cool white light at an intensity of 50 μeinstein/(m^2 s). Similarly prepared biomass was harvested, washed with deionized water, and freeze dried for free biomass (FBCS) nickel(II) biosorption studies.

2.2. Immobilizing material and technique

The reticulated loofa sponge was obtained on removing hard pericarp tissue of the ripened dried fruit of *L. cylindrica*. The sponge was cut into round pieces of approximately 2.5 cm diameter, 2–3 mm thick, soaked in boiling water for 30 min, thoroughly washed under tap water, and left for 24 h in distilled water, changed 3–4 times. The sponge pieces were oven dried at 70 °C, autoclaved for 20 min and soaked in Bold's medium for 5–10 min under aseptic conditions. Four pre-weighed loofa sponge pieces were transferred to 100 ml Bold's medium contained in 250 ml flasks. Each of these flasks was inoculated with 5 ml, 3–4-week-old stationary phase cultures of *C. sorokiniana* and incubated under similar conditions as those for developing inoculum biomass. The loofa sponge pieces were removed from the culture flasks, washed thoroughly with fresh culture medium to remove any free algal cells, transferred to 100 ml fresh medium and incubated under the same cultural conditions. The immobilized biomass (LIBCS) was harvested after 24 days, washed thoroughly with deionized water and freeze dried for further studies on metal biosorption. Quantity of the LIBCS was determined as the difference between constant dry weights of the loofa sponge, before and after immobilization. For scanning electron microscopy, samples of loofa sponge, free and loofa sponge-immobilized cells of *C. sorokiniana* cells were coated under vacuum with a thin layer of gold and examined by scanning electron microscope (Philips PSEM 501B).

2.3. Biosorption studies

Desired concentrations of nickel(II) solution were prepared by diluting standard nickel(II) stock solution ($Ni(NO_3)_2$, Merck) of concentration 1000 ± 2 mg/l. pH of the solution was adjusted to 5.0, unless otherwise stated using 0.1 M NaOH. Fresh dilutions were used for each biosorption study. The biosorption capacity of both FBCS and LIBCS was determined by contacting various concentrations (2.5–200 mg/l) of 100 ml nickel(II) solution in 250 ml flasks, with 100 ± 2.6 mg microalgal biomass. The nickel(II) solution, so incubated with algal biomass, was shaken on an orbital shaker at 100 rpm in tightly stoppered flasks at $25 \pm 2\,^{\circ}$C. FBCS was removed from metal solution by centrifugation at 5000 rpm for 5 min, whereas the LIBCS was separated from the solution by decantation. Residual concentration of nickel(II) in the metal supernatant solutions was determined using an atomic absorption

spectrophotometer (UNICAM-969). For the determination of rate of metal biosorption by both FBCS and LIBCS, the supernatant was analyzed for residual nickel(II) after the contact period of 5, 10, 15, 20, 30, 45, 60, and 120 min. The effect of pH on nickel(II) sorption by FBCS and LIBCS was determined by equilibrating the sorption mixture at different pH values of 2, 3, 4, 5, and 6. Metal-free solution and microalgal biomass-free metal solution containing only loofa sponge blanks were used as controls.

2.4. Reproducibility and data analysis

Unless indicated, the data shown are the mean values from three separate experiments. Statistical analysis of the data was carried out using the Duncan's new multiple range test [15]. The amount of metal ions adsorbed per unit free and immobilized biomass (mg metal/g dry biosorbent) was determined using the following expression:

$$q = \frac{V(C_i - C)}{M} \quad (1)$$

where q is the metal uptake (mg nickel(II)/g dry weight of algal biomass entrapped within loofa sponge), V is the volume of metal solution (ml), C_i the initial concentration of nickel(II) in the solution (mg/l), C is the residual concentration of nickel(II) in the solution at any time, and M is the dry weight of fungal biomass.

The Langmuir and Fruendlich equilibrium models were used for the evaluation of the adsorption data. Langmuir isotherm assumes monolayer adsorption, and is presented by the following equation:

$$q_{eq} = \frac{q_{max} b C_{eq}}{1 + b C_{eq}} \quad (2)$$

where q_{eq} and q_{max} are the equilibrium and maximum uptake capacities (mg/g biosorbent); C_{eq} is the equilibrium concentration (mg/l solution); and b is the equilibrium constant (l/mg).

The Freundlich model is presented by Eq. (3).

$$q_{eq} = K_F C_{eq}^{1/n} \quad (3)$$

where K and n are Freundlich constants characteristic of the system.

2.5. Desorption studies

For desorption studies analytical grade HCl, H_2SO_4, Na_2CO_3, EDTA, $NaHCO_3$, NH_4Cl, and CH_3COOH were used. For batch desorption experiments, a series of 250 ml Erlenmeyer flasks containing 50 ml desorption solution of known concentration was contacted with nickel(II)-loaded biosorbent (100 mg) at room temperature ($25 \pm 2\,°C$). The mixtures were agitated on orbital shaker at 100 rpm for 30 min. The LIBCS was removed and the supernatant was analyzed for nickel(II) released into the solution by atomic absorption spectrophotometer.

2.6. Biosorption–desorption cycles

The reusability of biosorbent is directly related to the application potential of biosorption technology. The same LIBCS, therefore, was reused in seven biosorption–desorption cycles to determine its reusability. For this purpose, 100 mg of LIBCS was contacted with 100 ml of 10 mg/l nickel(II) solution for biosorption and 50 ml of 75 mM HCl for desorption in 250 ml flasks, shaken on orbital shaker at 100 rpm and $25 \pm 2\,°C$. Each biosorption and desorption cycle was allowed 30 min of LIBCS–nickel(II) solution or LIBCS–desorbent agent contact for achieving sorption or desorption equilibrium. The initial and final nickel(II) concentration of the solution was recorded for each cycle. On the completion of every cycle, LIBCS was recovered by decantation, washed repeatedly with deionized water and transferred to nickel(II) solution for the next biosorption cycle.

2.7. Removal of nickel(II) from electroplating effluent

LIBCS performance for the removal of nickel(II) from authentic wastewater was investigated in a continuous flow fixed bed column bioreactors (2.7 cm column i.d., 30 cm column length) packed with 1.57 ± 0.063 g of LIBCS, packing height 28 cm. For nickel removal, authentic wastewater from electroplating industry was pumped upwards through the columns at a flow rate of 5 ml/min using peristaltic pump (Cole Parmer). Effluent samples were collected for every 500 ml of nickel(II) solution passed and analyzed for nickel(II) concentration. Biosorption saturation capacity of the column packed with LIBCS was considered reached at the stage at which no nickel(II) sorption occurred as indicated by the attainment of inlet–outlet nickel(II) equilibrium. The column bed was then rinsed by passing about 500 ml deionized water in an upward direction at the same speed as used for nickel(II) biosorption from the metal solution. The nickel(II) content was determined in all effluent fractions, including the deionized water rinse, collected from the column. Desorption was carried out by passing 700 ml of 75 mM HCl through the column bed in an upward direction at the flow rate of 5 ml/min.

3. Results and discussion

3.1. Preparation of LIBCS

Both visual and microscopic examination of loofa sponge discs revealed *C. sorokiniana* cells immobilization on the sponge fibers in 5–7 days of incubation. The sponge pieces were, nevertheless, continued to be incubated in the culture medium for further 3 days to allow complete and stable immobilization. The immobilized microalgal biomass was, thereafter, subcultured in fresh culture medium and maintained in batch culture for 24 days. The fibrous network of

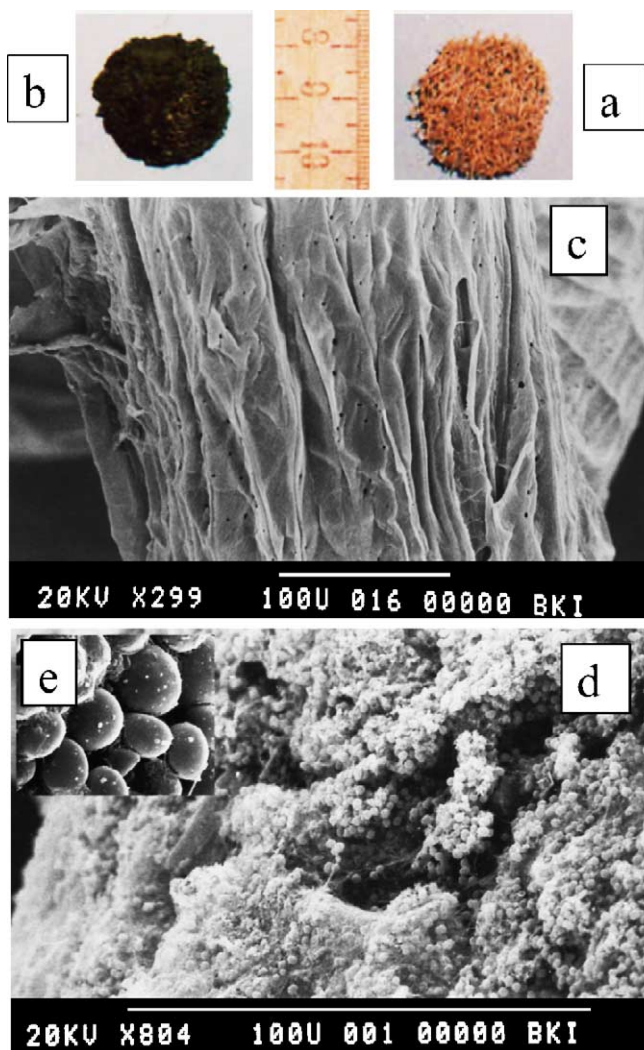

Fig. 1. Immobilization of *Chlorella sorokiniana* on the loofa sponge of *Luffa cylindrica*: (a) loofa sponge piece (naked); (b) sponge piece covered with immobilized biomass of *Chlorella sorokiniana* (LIBCS); scanning electron micrograph of (c) single thread of sponge, (d) *Chlorella sorokiniana* cells immobilized along sponge fibers, (e) enlarged portion of immobilized cells.

the sponge was noted to be covered by immobilized microalgal cells during this period (Fig. 1b). Representative scanning electron micrographs for the loofa sponge single fiber before and after the immobilization of *C. sorokiniana* are presented in Fig. 1c and d. The SEM micrograph of LIBCS revealed a uniform algal growth along the surface of the fibrous thread indicating that immobilized *C. sorokiniana* cells are not localized at a single point. This uniform distribution is an important criterion for the proper biosorption of heavy metal ions on the entire surface area of the immobilized algal cells. Thus, immobilization of the cells on the surface of loofa sponge threads could also provide additional advantages over the freely suspended algal cells. In free cultures, algal cells form individually distributed spherical clumps. This tight packing of algal cells could also lead to diffusional restriction and less adsorptive sites for heavy metal ions than the loofa sponge-immobilized cells. The amount of immobilized *C. sorokiniana* in the loofa sponge was 261 ± 22 mg/g of dry sponge. It was determined at the end of 24 days of growth and no increase in *C. sorokiniana* biomass was noted after this period.

3.2. Chemical and physical stability of LIBCS

The chemical and physical stability of biosorbent are important considerations in determining their operational life for wastewater treatment. Tests were, therefore, conducted to determine chemical and physical stability of LIBCS biosorbent before their use as a biosorbent in continuous flow wastewater treatment system. For the purpose, LIBCS were packed in columns and synthetic wastewater (pH 5.0) containing 1 mg/l of Ni(II), Pb(II), Zn(II), Cu(II), Cd(II), and Cr(III) was passed in an upward direction continuously for 20 days at a flow rate of 10 ml/min. No physical swelling or contraction, deterioration or significant weight loss of the LIBCS were observed during this period. Similarly, soaking of LIBCS in buffer of various pH (2.0–13.0 for 20 days) had no noticeable affect on the physical properties of the LIBCS. In contrast, alginate beads, the most widely used polymeric matrix for microorganisms for metal biosorption, have been reported to be stable only at pH 6–9 [16]. The LIBCS was also found to be stable in salt solutions. Thus, the LIBCS will perform better than hydrogel biosorbents in terms of their stability and reusability where a large amount of cell leakage has been reported during biosorption processing [17].

3.3. Biosorption characterization

3.3.1. Biosorption rate

The biosorption of nickel(II) by both FBCS and LIBCS was found to be extremely rapid reaching equilibrium in 20 and 15 min for FBCS and LIBCS, respectively. This rapid kinetics has significant practical importance as it will facilitate smaller reactor volumes ensuring efficiency and economy. From the Fig. 2, it is evident that loofa sponge without *C. sorokiniana* biomass adsorbed the nickel(II) far less than that of either FBCS or LIBCS, suggesting the role of *C. sorokiniana* biomass in the biosorption of nickel(II) from the solution. In comparison, an increase of 25.3% in the biosorption capacity of LIBCS was noted than the FBCS. The statistically significant lower uptake of nickel(II) by FBCS may be attributed to their aggregation due to electrostatic interaction between them, thus, reducing their three dimensional surface area for sorption. Raw, non-living free algal cells, as were FBCS cells used in present studies, tend to clump together [18]. Metal sorption efficiency of free mass of yeast cells and fungal hyphae has been reported to decrease due to reduction in distance between them, resulting in intracellular linkages between their reactive groups [19,20]. The structural microbarrier so created also limits accessibility of metal ions to the binding sites for adsorption through reduced diffusion [21]. Higher sorption of

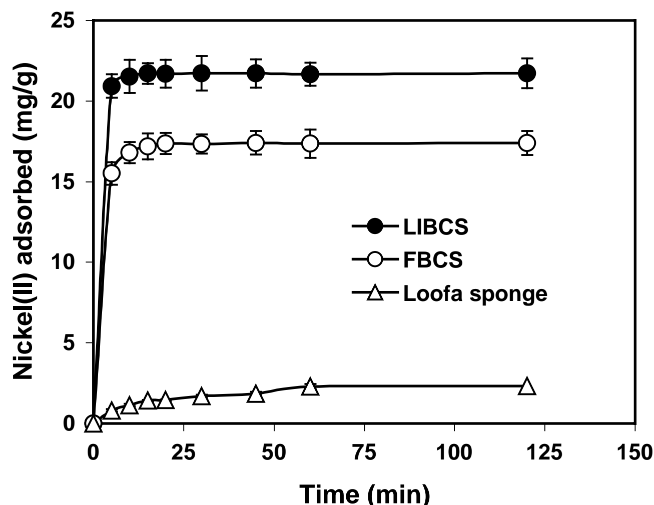

Fig. 2. Biosorption of nickel(II) from 25 mg/l solutions, pH 5.0, by 1 g/l microalgal cell biomass of *Chlorella sorokiniana* free (FBCS) or immobilized on loofa sponge (LIBCS) as related to the time of contact during orbital shaking at 100 rpm at 25 °C.

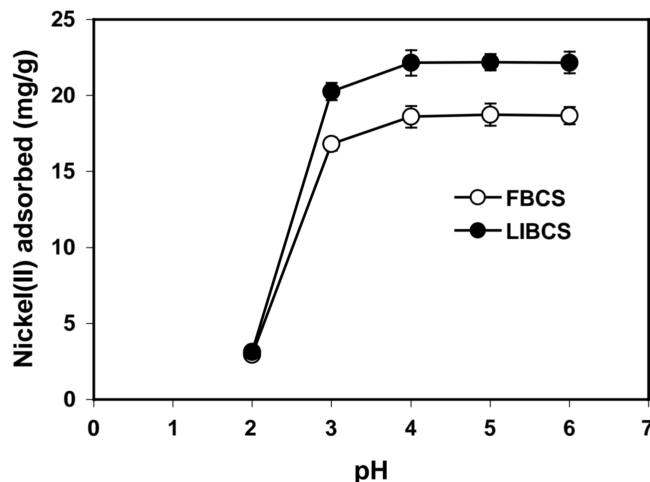

Fig. 3. Biosorption of nickel(II) from solutions of different pH, by 100 mg FBCS or LIBCS mixed in 100 ml 25 mg/l nickel(II) solution contained in 250 ml flasks and incubated on orbital shaker at 100 rpm at 25 °C.

nickel(II) by LIBCS, on the other hand, is evidently due to cell immobilization along the surface of the fibrous threads (Fig. 1d), little or no interaction with other immobilized cells in the biomass, no clumping, and the reticulated open network of immobilized matrix, together contributing to enhanced surface area and free access of the metal to sorption sites. In comparison with FBCS, the higher rate of nickel(II) removal by LIBCS further indicates that no diffusional limitations were presented as noted with immobilization of a mixture of organisms from activated sludge in hydrogels in which case significant decrease in the rate of metal sorption occurred [21]. In another study, 40% reduction in the sorption of lead(II), in comparison with free cells was noted when *Stichococcus bacillaris* was immobilized on silica gel [22]. Lopez et al. [23] also noted about 60% decrease in the rate of metal sorption by *Pseudomonas fluorescens* cells immobilized in agar beads, as compared with free cells. Surface immobilization of *C. sorokiniana* on individual threads of loofa sponge providing direct contact of biomass to metal solution is, therefore, better suited for biosorption than enclosed or beaded immobilization in polymeric gel structure.

3.3.2. Effect of pH

Metal biosorption is critically linked with pH. Not only different metals show different pH optima for their sorption but may also vary from one kind of biomass to others [24–26]. In order to establish the effect of pH on the biosorption of nickel(II) ions on to FBCS and LIBCS, the batch equilibrium studies at different pH values were carried out in the range of 2.0–6.0 (Fig. 3). The maximum adsorption of nickel(II) ions on both FBCS and LIBCS were observed at pH 4–5 and significantly decreased by reducing the pH values to 2.0. The increase in nickel(II) removal with increase in solution pH has also been observed for free algal biomass of *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Synechocystis* sp. [24] and immobilized cells of bacteria *Enterobacter* sp. [27] According to Low et al. [28] little sorption at pH 2 could be ascribed to the hydrogen ions competing with metal ions for sorption sites. This means that at higher $[H^+]$, the biosorbent surface becomes more positively charged, thus, reducing the attraction between biomass and metal ions. In contrast, as the pH increases, more negatively charged surfaces become available, thus, facilitating greater metal uptake [29]. It may be concluded that for both FBCS and LIBCS, biosorption plateau for nickel(II) was attained at the pH range of 4–5, being in general agreement with other reported in literature.

3.3.3. Effect of biosorbent concentration

One of the parameters that strongly affect the biosorption capacity is the concentration of the biosorbents. With the fixed nickel(II) concentration of 25 mg/l, the biosorption of nickel(II), in terms of percentage of metal adsorbed, increased with increasing quantity of FBCS (Fig. 4) and the highest nickel(II) uptake was observed at 1.0 g/l. A significant decrease in the uptake of nickel(II) by FBCS was noted after the biosorbent concentration was increased from 1 to 2.5 g/l. Conversely, an opposite trend was noted for LIBCS where an increase in nickel(II) uptake was continued with the increase of biosorbent concentration (Fig. 4).

The reduction in the nickel uptake by FBCS may be attributable to cell aggregation. Metal sorption efficiency of free biomass of yeast cells and fungal hyphae has been reported to decrease due to cell aggregation (where cells tend to clump) and reduction in distance between them with increasing free cell concentration [20,30]. As far as higher sorption of nickel(II) by LIBCS is concerned, it is evidently due to cell immobilization along the surface of the fibrous threads which allowed little or no interaction with the rest

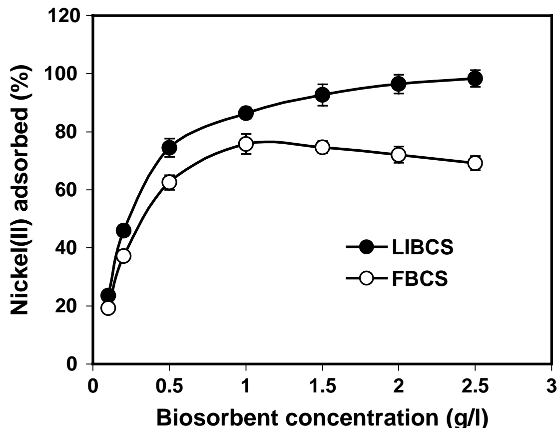

Fig. 4. Effect of biosorbent concentration on metal sorption in 25 mg/l solution of nickel(II) at pH 5.0 contacted with 0.1–2.5 g/l of FBCS or LIBCS.

of the immobilized cells in the nickel(II) solution and so did not permit cells to clump. Therefore, the binding sites on the cell wall have maximum accessibility to nickel(II) and this attributed to increased uptake of nickel(II) with increasing immobilized cell concentration, as long as the latter is not saturated.

3.3.4. Effect of initial metal concentration

Nickel(II) biosorption capacities of LIBCS and FBCS were presented as a function of initial concentration of metal ions within the aqueous biosorption solution in Fig. 5. The initial concentration was changed in the range of 2.5–200 mg/l. The amount of nickel(II) ions adsorbed per unit mass of the biosorbent increased with the initial concentration of metal ions. In order to reach the plateau values, which represent saturation of the active sites on the biosorbent, in other terms, to obtain the maximum biosorption capacity, the initial concentration of nickel ions was increased up to 200 mg/l. As can be seen from Fig. 5, the amount of nickel(II) ions adsorbed on plain loofa sponge was 6.1 mg/g. Maximum biosorption capacity of nickel(II) for FBCS and LIBCS was found as 48.08 and 60.38 mg/g, respectively.

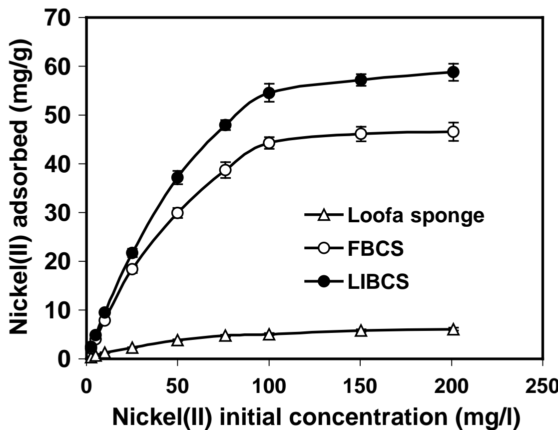

Fig. 5. Effect of initial concentration of metal ions on biosorption of nickel(II) by loofa sponge, FBCS and LIBCS; 100 ml solution of nickel(II) (2.5–200 mg/l; pH 5.0) mixed with each biosorbent at 100 rpm at 25 °C.

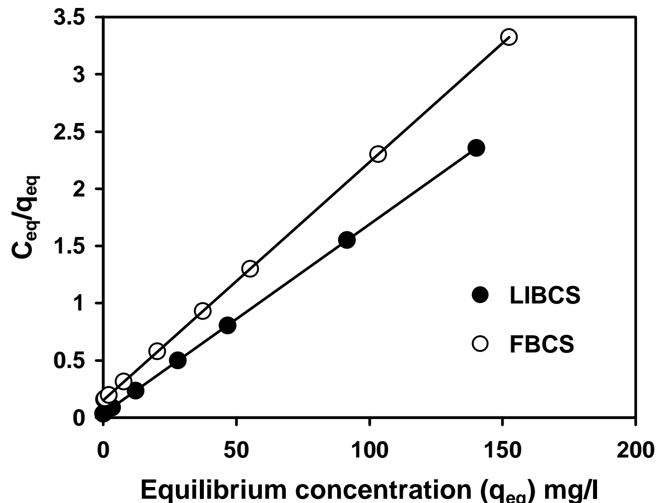

Fig. 6. Linearized Langmuir adsorption isotherms for nickel(II) sorption by FBCS and LIBCS based on specific quantity (q_{eq}) sorbed and quantity of nickel(II) in solution at equilibrium (C_{eq}).

3.3.5. Adsorption isotherms

In order to optimize the design of the sorption system it is important to establish the most appropriate correlation for the equilibrium curves. Two kinds of several isotherms equations have been applied for this study, Langmuir and Freundlich isotherms. The linearized Langmuir and Freundlich isotherms of nickel(II) are shown in Figs. 6 and 7. The Langmuir and Freundlich adsorption constants evaluated from the isotherms with the correlation coefficients are given in Table 1. In view of the values of linear regression coefficients in the table, the Langmuir model exhibited a little better fit to the sorption data of both FBCS and LIBCS than the Freundlich model in the studied concentration ranges.

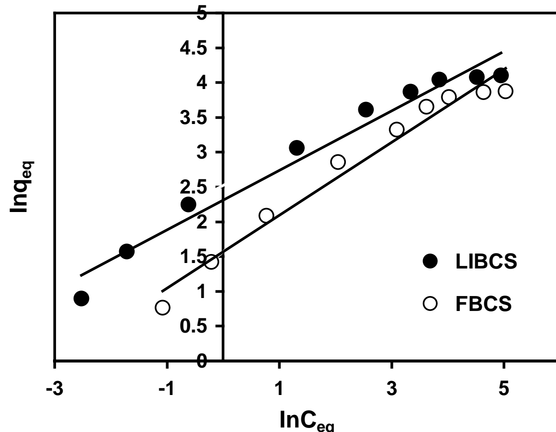

Fig. 7. Linearized Freundlich adsorption isotherms for nickel(II) sorption by FBCS and LIBCS.

Table 1
Isotherm model constants and correlation coefficients for biosorption of nickel(II) from aqueous solution

Biosorbents	Langmuir			Freundlich		
	q_{max} (mg/g)	b (l/mg)	R^2	K_F	n	R^2
FBCS	45.87	0.135	0.991	4.28	1.91	0.968
LIBCS	59.58	0.546	0.993	10.08	2.35	0.964

However, the Freundlich model also seemed to agree well with the experimental data of the nickel(II) considering that obtained linear regression coefficients are greater than 0.96. The magnitude of K_F and n; the Freundlich constants, confirmed the higher potential of nickel(II) removal for LIBCS than FBCS. In the case of Langmuir isotherm model, the higher value of q_{max} and b of LIBCS further indicates the superiority of this newly developed biosorbent over the FBCS. Table 2 shows a comparison between the results of this work and others found in the literature. The values of nickel(II) specific uptake found in this work were significantly higher, with one exception [31], than reported elsewhere. The comparison of sorption capacities of LIBCS used in this study with those obtained in the literature shows that the LIBCS is the most effective for the removal of nickel(II) from aqueous solution.

3.3.6. Precision and reproducibility of LIBCS

For continuous supply of LIBCS during this characterization study, micro alga *C. sorokiniana* was grown, immobilized, and harvested in regular and carefully monitored different batches. A batch of 48 flasks was cultivated and collected monthly with each batch yielding 192 LIBCS discs. The LIBCS so obtained were then freeze dried in different lots. Therefore, it is important to assess the reproducibility and precision between the LIBCS produced in different lots and batches. For the purpose, different sets of biosorption experiments, using LIBCS from different lots and batches were designed and nickel(II) removal capacity of each set of experiment was determined (Table 3).

Table 3
Reproducibilty and precision of experimentally determined nickel(II) capacity for LIBCS produced in different cultivation batches and freeze drying lots

	Lot to lot reproducibility and precision				Mean	S.D.	R.S.D. (%)
	A1	A2	A3	A4			
Set-1	56.11	56.01	54.94	57.08	56.03	0.87	1.56
Set-2	57.23	56.96	56.34	56.52	56.75	0.39	0.70
Set-3	55.70	56.49	57.41	55.84	56.37	0.76	1.36
	Batch to batch reproducibility and precision						
	A	B	C	D			
Set-1	56.64	58.12	52.61	55.48	55.71	2.33	4.18
Set-2	55.93	57.27	54.52	53.31	56.00	2.29	4.09
Set-3	58.27	57.56	53.01	53.62	55.62	2.68	4.82

Uppercase letters were used to designate the algal batch and associated number represent the drying lot. For example A1 and A2 represent the same batch of LIBCS dried in different lots. Excellent precision and reproducibility in nickel(II) removal capacity was noted between LIBCS produced in different drying lots and batches of cultivation with the greatest relative standard deviation (R.S.D.) represented as 1.6 and 4.8%, respectively. This suggests that LIBCS biosorbent produced in different batches/lots are relatively homogenous and the nickel(II) removing capacity of LIBCS under the conditions investigated is reproducible.

3.4. Desorption characterization

3.4.1. Recovery of adsorbed nickel(II) from LIBCS

A preliminary evaluation of the desorbing agents was carried out under batch experimental conditions and desorption efficiencies were compared in Fig. 8. The use of Na_2CO_3, $NaHCO_3$, and NH_4Cl solutions resulted in only limited amount of nickel(II) desorption, less than 30%. Acetic acid showed a desorption efficiency of about 49.6%. Deionized

Table 2
Comparison between the nickel(II) (mg/g LIBCS) removal by LIBCS [tw] and others found in the literature

Biosorbent	Operational conditions				q_{eq} (mg/g)	Ref.
	pH	T (°C)	C_i (mg/l)	M (g/l)		
Chlorella vulgaris	5.0	25	100	1.0	42.3	[24]
Scenedesmus obliquus	5.0	25	100	1.0	18.7	[24]
Synechocystis sp.	5.0	25	100	1.0	15.8	[24]
Phormidium laminosum	5.0	30	100	1.0	22.23	[33]
Ascophyllum nodosum	6.0	25	200	n.a.	70	[31]
Focus vesiculosus	3.5	25	200	n.a.	17	[31]
Saccharomyces cerevisiae	5.0	25	10–200	1.0	46.3	[34]
Polyporous versicolor	5.0	25	50–500	n.a.	47.0	[35]
Rhizopus arrhizus	6–7	n.a.	10–600	3.0	18.7	[30]
Arthrobacter sp.	5.5	30	150	1.4	13.0	[36]
Chlorella sorokiniana	5.0	25	200	1.0	60.57	[tw]

n.a.: not available.

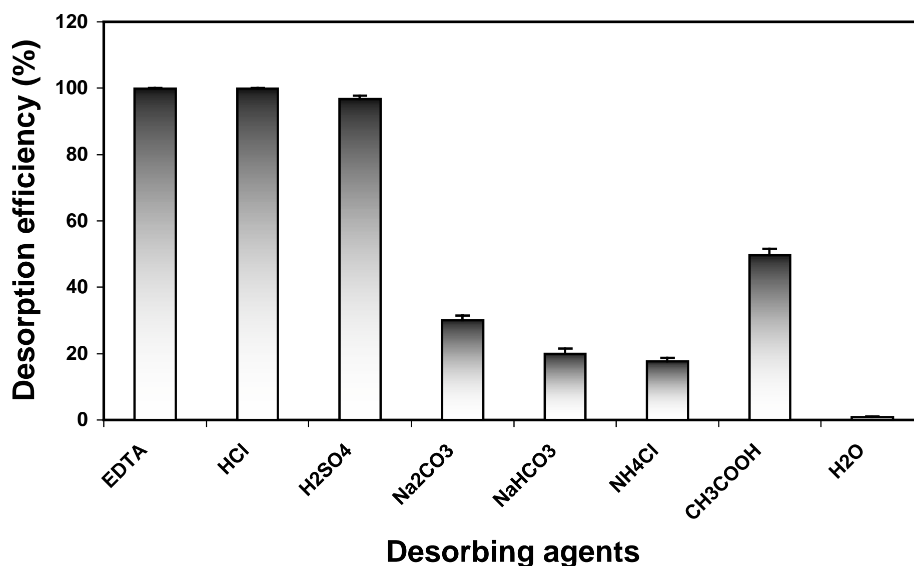

Fig. 8. Desorption efficiencies of nickel(II) from LIBCS by using different desorbing agents (concentration of desorbing agents: 0.1 M, LIBCS: 1 g/l).

water (control) showed insignificant nickel(II) desorption (0.81%). On the other hand, mineral acids, HCl, and H_2SO_4, resulted in high recovery efficiencies of about 99.96 and 96.74%, respectively. In addition, complexing agent EDTA also resulted in high recovery efficiency of 99.97%. The low desorption efficiencies of Na_2CO_3, $NaHCO_3$, and NH_4Cl can be attributed to the fact that divalent cations (e.g. nickel(II)) have greater affinities for the negative-charged sites on the biosorbents than on monovalent cations (e.g. K^+, Na^+). EDTA had a similar regeneration efficiency to that of HCl. This can be attributed to its strong complexing ability to nickel(II). However, it has the disadvantage of high cost when compared to HCl. It is also difficult to recover the metal ions from EDTA solution as the stability of metal–EDTA complex is very high. For H_2SO_4, a possible precipitation reaction may take place within the biosorbent particle between the calcium ion and the other heavy metal ions that may be present in the system. Overall, HCl was selected as the optimal eluting agent for the system studied. Sorption experiments were conducted to establish the optimum concentrations for the desorption agent HCl. Biosorbents loaded with nickel(II) were contacted with HCl solutions with different concentrations. The amount of nickel(II) released back into the solution was then determined and expressed as a desorption efficiency as shown in Fig. 9. The results show that increasing the concentrations of HCl increased the desorption capacity. However, beyond some optimum values, desorption efficiency plateau. From. Fig. 9, it can be seen that a concentration of 75 mM HCl or higher can remove more than 98.0% of adsorbed nickel(II). Therefore, the lower concentration of 75 mM HCl was used for elution in subsequent studies.

3.4.2. Adsorption–desorption cycles

Reusability of a sorbent is of crucial importance in industrial practice for metal removal from wastewater. In order to

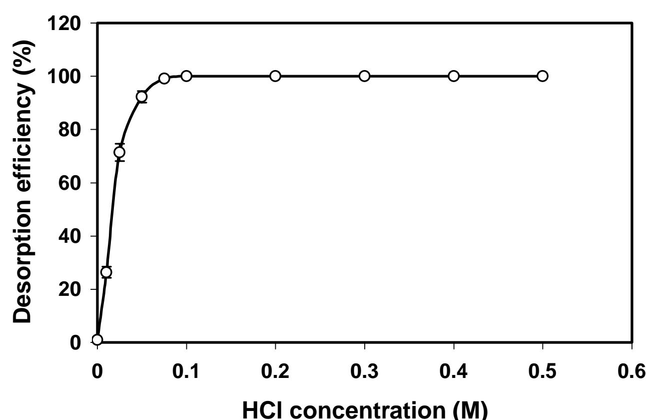

Fig. 9. Effect of HCl concentration on the desorption of nickel(II) from LIBCS.

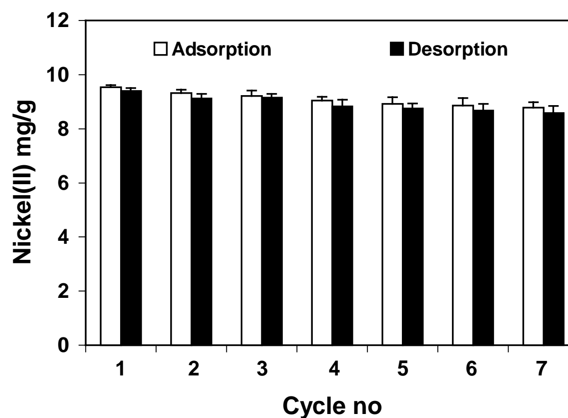

Fig. 10. Biosorption–desorption of nickel(II) by LIBCS in seven consecutive cycles.

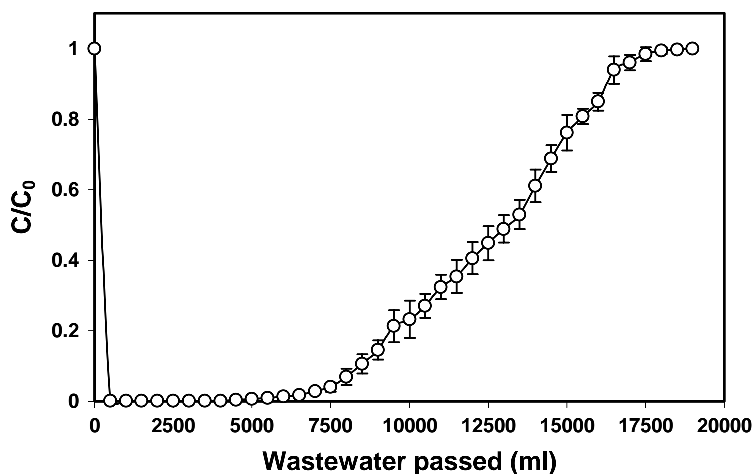

Fig. 11. The performance of LIBCS in fixed bed column bioreactor for the treatment of authentic wastewater from electroplating discharge containing nickel(II) at the concentration of 5.25 mg/l.

show the reusability of the LIBCS, adsorption–desorption cycle of nickel(II) was repeated for seven times using the same preparation (Fig. 10). The nickel(II) ions adsorbed onto LIBCS were desorbed with 75 mM HCl in batch system. Approximately 98% of the adsorbed nickel(II) ions were desorbed from the LIBCS. It was observed that the nickel(II) uptake for cycles 2–7, although slightly lower than that for cycle 1, were reasonably consistent irrespective of the number of cycles (Fig. 10). The total decrease in biosorption efficiency of LIBCS after seven cycles was about 7.6% which shows that the LIBCS has good potential to absorb metal ions from aqueous solution.

3.5. Treatment of electroplating effluents

To evaluate the potential performance of LIBCS for the removal of nickel(II) from authentic wastewater, the LIBCS were packed in bench scale columns with a diameter of 2.7 cm and length of 30.0 cm. For the test, the electroplating rinse effluent (nickel(II) concentration 5.25 mg/l, pH 6.7) from nickel(II)-plating line of PECO bicycle industries, Lahore, Pakistan, characterized by high amounts of Na^{2+} (115.39 mg/l), Mg^{2+} (73.27 mg/l), and Ca^{2+} ions (46.52 mg/l) was passed through the column in an upward direction at a flow rate of 5 ml/min. The nickel(II) loading curve (Fig. 11) showed an excellent, clear (i.e. 100% removal) zone before the breakthrough point. Approximately 5.5 l of electroplating effluent was treated completely before breakthrough occurred. In the loading stage, a total of 64.81 ± 1.86 mg of nickel(II) was accumulated in the column. The number of 64.81 mg nickel(II) was obtained by numerical integration of the whole breakthrough curve. Thus, the nickel(II) biosorption capacity of the LIBCS in the column operation is 43.11 ± 1.55 mg of nickel(II)/g LIBCS. The data presented in Fig. 11 clearly shows that the nickel(II) can be efficiently removed from the electroplating effluent by the LIBCS packed column, although the presence of some divalent metal ions such as Na^{2+}, K^{2+}, Ca^{2+}, and some other components in the wastewater have inhibited the nickel(II) binding by the LIBCS as compared to the nickel removal from aqueous solution containing nickel as a single metal in batch studies. Similar reduction in the removal of heavy metals was noted when other microbial biomass were used to remove metal ions from industrial effluents [17,32]. Data from the corresponding regeneration curve again showed promising (94%) nickel(II) recovery from the loaded column by 75 mM HCl.

4. Conclusions

1. Loofa sponge is an effective immobilization carrier for the entrapment of micro alga *C. sorokiniana* to produce LIBCS.
2. LIBCS showed an excellent potential for the removal and recovery of nickel(II) from aqueous solution containing nickel(II) and authentic wastewater.
3. No loss to biosorption capacity of LIBCS was found due to the presence of loofa sponge, in addition as compared to FBCS an increase of 25.58% was noted in the nickel(II) biosorption capacity of LIBCS.
4. The sorption of nickel(II) by the LIBCS was found to be influenced by operational conditions particularly medium pH and the concentration of biosorbent and metal ions in the medium.
5. LIBCS showed an excellent physical and chemical stability as no significant leakage or breakage of immobilized biomass was observed during their repeated use in biosorption–desorption operations.
6. LIBCS could be regenerated and reused at least for seven biosorption and desorption cycles of nickel(II) with a little decrease in the metal uptake capacity of the biomass.
7. Loofa sponge is an inexpensive, easily available biomaterial and LIBCS biosorbent can be easily produced by

inoculating a growth medium containing the loofa sponge pieces with an appropriate microbial inoculum, without any prior chemical treatment. In contrast, production of beads from polymeric matrices for commercial application is expensive, laborious and requires sophisticated equipment.

8. The data from the present study showed that LIBCS has promising potential in remediation of nickel(II) laden effluents.

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