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Cu^{2+} Removal and recovery by *Spi*SORB: batch stirred and up-flow packed bed columnar reactor systems

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Abstract The biosorption of Cu^{2+} by free and poly acrylamide gel (PAG) immobilized *Spirulina platensis* (*Spi*SORB) was characterized under batch and continuous packed bed columnar reaction systems. The biosorption of Cu^{2+} was shown to be highest at pH of 6.0 for both types of biomass. The PAG immobilization process did not interfere with the Cu^{2+} binding sites present on biomass leading to cent percent (ca. 250 mg g^{-1} of dry biomass) retention of biosorption as compared to free cells. Transmission electron microscopy on Cu^{2+} localization revealed that majority of metal is being sequestered by the cell wall only. The infrared spectrum of metal treated *S. platensis* biomass indicated the possible involvement of amide, amino, and carboxyl groups in metal binding. Up-flow packed bed columnar reactor containing 2.0 g of PAG immobilized *S. platensis* shown a maximum of 143-fold volume reduction factor at the residence time of 4.6 min for Cu^{2+} alone and found to decrease dramatically when Zn^{2+} is present in a bimetallic solution.

Keywords Biosorption · Heavy metals · *Spi*SORB · PAG cubes · Column runs

Introduction

The aqueous discharges emanating from industrial processes such as mining, smelting, metal-plating, ore processing activities, and energy production processes contain dissolved heavy metals that can generate significant environmental problems [1]. The use of microbial biomass for the detoxification of industrial effluents for environmental protection and recovery of valuable metals through biosorption offers a potential alternative to existing technologies [2, 3]. Biosorbents in the form of biomass powder may be utilized directly as additives in a stirred-tank reactor followed by solid–liquid separation facilities, or the biomass may be immobilized in a solid particulate matrix and packed in a flow-through columnar reactor [4]. Immobilized biomass have shown greater potential in packed—or fluidized—bed reactors, in terms of control of particulate size, efficient regeneration of biomass, ease of biomass separation from effluent, and minimal clogging under continuous-flow conditions. In addition, easy operation of repeated biosorption–desorption cycles with biosorbent beads makes the biosorption process potentially more economic and competitive. Physical entrapment of organisms inside a polymeric matrix is one of the most widely used techniques for whole cell immobilization [5].

Cyanobacteria (blue-green algae) are a diverse group of prokaryotes, exhibiting versatile physiology and wide ecological tolerance that contribute to their competitive success over a broad spectrum of environments [6]. These oxygen-evolving organisms quickly respond and adapt to stress conditions in general [7] and heavy metals in particular [8]. They have developed natural methods of resistance towards heavy metals, viz., a reduction in metal intake [9], extracellular sequestration [10], localization/compartmentalization inside the cell [11] or energy-dependent efflux [12], etc. *Spirulina platensis* is a cyanobacterium that gained attention because of its anti-radiation and anti-mutation properties [13]. In the present study, we report our findings on the optimization of essential process parameters for biosorption of Cu^{2+} by the *S. platensis* cells immobilized in polyacrylamide gel (PAG; hence forth called as *Spi*SORB) using packed bed columnar reactor. Due to covalently linked polymatrix nature PAG is believed to be more chemically stable in wastewater than the ionically cross-linked polymers such as calcium alginate gel [14]. In process applications, the most effective use of the reactor

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volume is a fixed bed column that also optimizes the concentration difference known to be the driving force in sorption process. In addition to that, the recycling of metal recovery is important in case of precious metals, whereas the recycling of biomass is essential for minimizing the costs of solid waste disposal.

Materials and methods

Strain and growth conditions

The cyanobacterium, *S. platensis* 730, was obtained from National Facility for Blue Green Algae, IARI, New Delhi. The axenic culture was grown in the medium described by Ogawa and Terui [15] and maintained in the culture room illuminated with cool day light fluorescent tubes (14.4 W m^{-2}) under a 16-h light/8-h dark cycle at 24 ± 1°C. For preparing lyophilized biomass, exponentially grown cells (7-day old) were harvested by centrifuging at 3000 rpm for 10 min before washing them with deionized distilled water followed by freeze-drying using *MAXI lyo* (Heto, Germany).

Preparation of polyacrylamide gel cubes

The lyophilized biomass was washed thrice with deionized distilled water and re-suspended in 20 ml sterile water (an aliquot of this cell suspension was dried at 105°C for determination of cell dry weight) supplemented with 2.5 g acrylamide monomer and 0.25 g N,N′-methylenebisacrylamide as a cross-linking agent. The polymerizing reaction was initiated by adding 2.5 ml of a 2.5% (w/v) solution of potassium per sulphate, and accelerated by adding 2.5 ml of a 5% (w/v) solution of 3-dimethylaminopropionitrile. The suspension was shaken gently until the gel began to set and was then refrigerated at 4°C throughout polymerization to prevent thermal damage to the cells. The gels were prepared in a closed rectangular tray, made up of acrylic sheet, having openings at two sides. When the gel had set, these two openings were unlocked and the gel was cut in to 9 mm^3 cubes. Cell free cubes were also prepared following the similar procedure described above. To optimize the biomass loading into PAG cubes, increasing concentrations of *S. platensis* lyophilized biomass (25–300 mg ml^{-1} of acrylamide, i.e. 16.2–69.8% of biomass loaded in PAG cube) were tested. Such cubes were used in stability and Cu^{2+} uptake experiments. The stability of the PAG cubes was tested by incubating eight cubes in 100 ml of metal solution for 72 h on rotary shaker under culture room conditions. The leakage of biomass from squares was examined by testing the ambient metal solution by plating 1 ml solution on agar medium.

Metal sorption experiments

Metal solutions were prepared by dissolving nitrates in deionized distilled water acidified with nitric acid as described by Raveender et al. [8]. Sorption experiments with varying initial metal concentrations (50–800 mg l^{-1}) were carried out with PAG square containing 27.9% (w/w) of biomass. The pH of the solution was adjusted by adding 0.1 N HCl or NaOH; this was maintained at pH 6.0 unless otherwise specified. All the flasks were sealed to minimize evaporation and shaken at 180 rpm on rotary shaker for the desired period under cool day light fluorescent tubes.

Following equilibration, the solutions were vaccum-filtered through 0.45 μm cellulose membrane filters. Metal concentrations in the filtrate were determined by Atomic absorption spectrophotometer (*A Analyst* 300, Perkin-Elmer, UK). Each sample was analyzed in triplicate against procedural blanks. The metal sorption by cyanobacterial biomass entrapped in PAG square was calculated by deducting the values obtained for biomass-less control squares. The metal sorption is calculated and expressed using the equation

$$Q = (C_i - C_f)\, V/M.$$

Where Q is the specific metal uptake (mg g^{-1} dry weight of biomass), V the volume of the metal solution (l), C_i the initial concentration of the metal in the solution (mg l^{-1}), C_f the final concentration of the metal in the solution (mg l^{-1}), and M is the dry weight of the biomass (g).

Desorption of bound Cu^{2+}

The desorption of Cu^{2+} by different mineral acids, organic acids, inorganic salts, and chelating agent (listed in Table 3) were studied by treating the metal loaded biomass as described by Hu and Reeves [14] for uranium recovery. The *S. platensis* cells loaded with 78 mg Cu^{2+} g^{-1} were washed with deionized distilled water through repeated centrifugation before incubating with desorbents solution for 10 min. The cells were separated from the reaction mixture through vacuum filtration using 0.45 μm cellulose membrane filter and washed with deionized distilled water. The amount of metal present in washable fraction was added to the final values. The metal concentration in the filtrate was measured using Atomic absorption spectrophotometer as described earlier and expressed as percent desorption of metal absorbed by the cells. The optimal time required for Cu^{2+} desorption was determined by drawing the samples at different time intervals (1–10 min) from the reaction mixture followed by its analysis for percent desorption.

Transmission electron microscopy

Transmission electron microscopic study was conducted according to the method described by Figueira et al. [16]. The *S. platensis* cells exposed to Cu^{2+} (600 mg l^{-1} for

2 h) were concentrated by centrifugation, washed several times with Na-phosphate buffer (0.1 M, pH 7.4) and fixed by submerging them in 2.5% (w/v) glutaraldehyde-Na phosphate buffer for 2 h at room temperature. The samples were washed repeatedly with the same buffer and dehydrated in a graded ethanol series (50–100%, w/v) and acetone (100%, w/v). Unstained ultrathin sections cut by a Reichart Ultracut E Ultra microtome (Reichart, Germany) with diamond knives and mounted on 100 mesh aluminum grids. Sections were examined and compared with metal free control by loading them in formvar carbon-coated grid in a Philips CM 100 transmission electron microscope at 100 kV.

Study with infra red spectroscope

To obtain a qualitative and preliminary analysis of the main chemical groups present on the cell wall and its contents, an infrared (IR) analysis in solid phase was performed on lyophilized biomass in a KBr disk using a JASCO IR report-100 Spectrometer. The IR spectra obtained at 4000–400 cm^{-1} range was used in examining the biomass before and after metal treatment (exposed to 600 mg l^{-1} of Cu^{2+} for 5 h).

Packed bed columnar reactor

The dimensions of the glass column used in the present biosorption study were 32 cm in length with an internal diameter of 2.3 cm. The column and its fittings were washed with 10% HNO_3 followed by thorough-rinsing with deionized distilled water before the experiment. The column was loosely packed with 320 PAG squares containing approximately 2.0 g of lyophilized *S. platensis* biomass (27.9% w/w). Influent solution contained either Cu^{2+} alone or a mixture of Cu^{2+} and Zn^{2+} (in the ratio of 1:22, in view of a typical electroplating industrial wastewater [17]) were prepared using deionized distilled water (pH 5.90 ± 0.05). For column regeneration, 10 mM Ca $(NO_3)_2$ was pumped and samples were collected every 1–2 min for analysis. Owing to its highest desorbing efficiency found during batch studies, 10 mM Ca $(NO_3)_2$ (pH 6.1) was selected and used in further studies.

Results and discussion

Effect of ambient pH

The specific and non-specific uptake of metal ions by the cell is a pH-dependent phenomenon [18] as hydrogen ion concentration is known to regulate metal toxicity and uptake in microbes and in algae. The Cu^{2+} uptake pattern by free and immobilized forms of *S. platensis* biomass showed a similar pattern of regular increase in uptake with increasing pH in the range of 3.0–6.0 reaching the maximum at pH 6.0 followed by a decline at pH range beyond 6.0 and above (Fig. 1). The poor metal uptake at low pH (3.0) and increase in uptake with increasing pH from 3.0 to 6.0 is in agreement with the earlier report on U uptake in *P. aeruginosa* CSU strain where the pH profile of uptake followed a similar increase in metal loading with increasing pH [19]. In general pH-dependent metal uptake could be the result of ionic attraction between metal ion and the cell surface as observed by Mallick [20] for Cu and Ni biosorption by *Chlorella vulgaris* and enhanced negative charge on the cell surface as described by Al-Asheh and Duvnjak [21] for Cu^{2+} sorption by *Aspergillus carbonarius* biomass. It has been reported that under low pH conditions the cell surface becomes positively charged leading to competition between metal ions and hydrogen (H^+) as well as hydronium (H_3O^+) ions, reducing the affinity between biomass and metal ions [22].

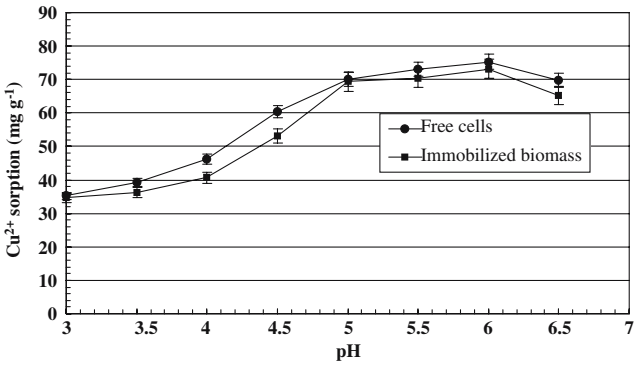

Fig. 1 Effect of ambient pH on Cu^{2+} sorption by free and immobilized *S. platensis* biomass; the free cells or cells immobilized in PAG matrix was incubated in the metal solution of different pH for 24 h under culture room conditions before estimating the Cu^{2+} sorption as described in experimental section

Optimization of biomass loading in to PAG cubes vis-à-vis stability and metal biosorption

The *Spi*SORB material was examined for its stability in terms of mechanical strength and Cu^{2+} biosorption by increasing biomass concentrations (25–300 mg ml^{-1}). The results indicate that PAG cubes were quite stable in

Table 1 Effect of biomass loading on stability and Cu^{2+} sorption by *S. platensis* cells immobilized in poly acrylamide gel (*PAG*) cubes

Biomass loaded in PAG cubes (% dry wt.)	Cu^{2+} sorption (mg Cu^{2+} g^{-1} biomass)	Cube stability (number of colonies appearing on agar plates)
16.2	32.7 ± 2.1	–
27.9	45.4 ± 3.4	–
43.8	37.0 ± 1.4	–
61.0	12.1 ± 2.4	10
69.8	8.6 ± 1.1	14

their mechanical strength until they were loaded with 43.8% (w/w) of biomass basis. A higher density of biomass in a cube showed a leakage of cells in to the reaction vessel as few colonies appeared on agar plates at the biomass loading beyond 43%. The maximum sorption of Cu^{2+} was shown by the cube having 27.9% (w/w) of biomass loading (Table 1). Thus the PAG cubes loaded with 27.9% biomass with good mechanical strength and optimal Cu^{2+} sorption capacity were used in subsequent experiments. Biomass loading rates into matrix vis-à-vis stability of the cubes influences the sufficient porous channeling leading to metal ion transport to the core of the matrix [23]. On the other hand, negligible metal uptake shown by empty PAG cubes may be due to presence of amino groups in the gel [24]. Thus, *Spi*SORB material retained same efficiency of Cu^{2+} uptake as compared to free biomass as observed by other workers for silica immobilization of *Nostoc muscorum* [25].

Effect of external Cu^{2+} concentration

The biosorption capacity of free and PAG immobilized *S. platensis* was evaluated by conducting experiments at varying metal concentrations (50–800 mg l^{-1}). Sorption performance by two forms of *S. platensis* biomass in terms of equilibrium isotherms showed a similar pattern of Cu^{2+} sorption. As the external concentration of Cu^{2+} increased the biosorption by both types of biomass increased and the maximum attainable biosorption was found to be approximately 250 mg Cu g^{-1} of dry biomass for both free and PAG immobilized biomass (dry), respectively (Fig. 2). The maximum experimental Cu^{2+} loading capacities found in this study are highest ever reported to date with algal biosorbents [26]. This could be partly due to the protein-richness (>70% of dry weight) of the *Spirulina* biosorbent. The *Spi*SORB retained same efficiency of Cu^{2+} sorption even at high Cu^{2+} concentration indicating that the PAG immobilization process does not inactivate or interfere with the metal binding sites of the biomass as observed by other workers for polyurethane hydrogel immobilization [14]. The external metal concentration dependency of biosorption clearly suggests that when all of the sites at the cell wall are occupied, internalization of metal can begin. Several independent studies have concluded that biosorption by fungus, algae, and peat moss occurs by an ion-exchange mechanism. Since the cyanobacteria used in this study were not treated in any way, it is assumed that protons occupy the external binding sites therefore biosorption would occur by the replacement of protons by free metallic ions. Further, the relationship between metal equilibrium sorption and residual concentration was studied by examining the closeness of fit of Langmuir and Freundlich models. Although both the models could moderately represent sorption process, the data showed a preference toward Freundlich model (Fig. 3) thereby showing the possibility of a single-layer deposition of the metal on the cell wall of *S. platensis* biomass as reported for Zn by *Rhizopus arrhizus* [18]. Values of respective sorption constants (adsorption capacity and adsorption intensity) and correlation coefficients (r^2) were derived from linearized isotherms and are shown in Table 2.

Kinetics of metal biosorption

The time-dependent Cu^{2+} sorption by free and PAG immobilized forms of *S. platensis* was examined by subjecting the biomass (50 mg) to 100 mg l^{-1} (a subsaturating concentration) of Cu^{2+} solution. The Cu^{2+} sorption by both forms of biomass indicate that the process involves two distinct steps with an initial rapid binding of metal followed by slow, secondary, and residual deposition of metal as reported earlier by our group for Cu^{2+} uptake in free living *Nostoc calcicola* [12]. The similar results have also been obtained for Pb, Cd, and Zn by *Citrobacter* [27] which are attributed to

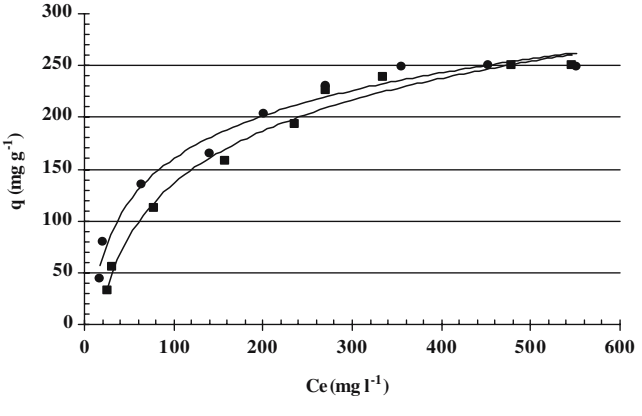

Fig. 2 Equilibrium isotherm of Cu^{2+} sorption by free and PAG immobilized *S. platensis*; free cells (*filled circles*), immobilized biomass (*filled squares*)

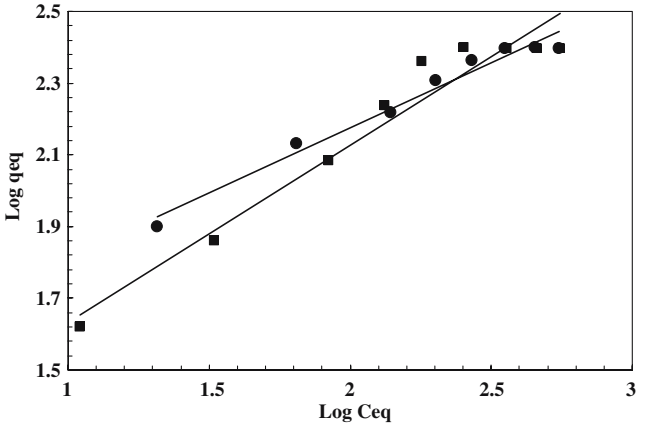

Fig. 3 Freundlich isotherm for Cu^{2+} biosorption by free and PAG immobilized *S. platensis*; free cells (*filled circles*), immobilized biomass (*filled squares*)

Table 2 Metal loading and Freundlich constants resulting from equilibrium sorption studies of Cu^{2+} by *S. platensis* biomass

Type of biomass	Q_{max} (mg g^{-1} dry wt.)	Adsorption capacity (k)	Adsorption intensity ($1/n$)	r^2
Free	250.3	1.45	0.36	0.9731
PAG Immobilized	243.8	1.14	0.49	0.9477

the faster solid–liquid contact, followed by residual and much slower additional metal deposition. The initial phase resulted into ca. 76 (62 mg g^{-1}) and 68% (51 mg g^{-1}) of total biosorption by free and PAG immobilized *S. platensis*, respectively. Initial rapid binding step was completed with in 1–2 min in the case of free biomass whereas it lasted for 10–12 min for immobilized form (data not shown). However secondary residual deposition of metal continued for 2 h in both the cases followed by a saturation (Fig. 4). The first step of rapid binding have shown to contribute 77.1–67.9% of the total metal sorption for free and immobilized biomass, respectively.

Desorption of bound Cu^{2+}

From the process point of view, desorption of bound metal resulting in to the recovering metal as well as regenerating the biomass enables the reuse of the biomass in repeated metal uptake and elution cycles. Due to the week nature of metal-binding at the cell wall, it is possible to detach the bound metal from the biomass by using various mineral acids, inorganic salts, and organic acids [14]. The desorption efficiency (expressed in percent ratio of metal desorption and uptake) of mineral acids was found to be maximum followed by inorganic salts, chelating agent, and organic acids (Table 3). The nitric acid showed maximum desorption (94.6%) in the mineral acid category followed by hydrochloric and

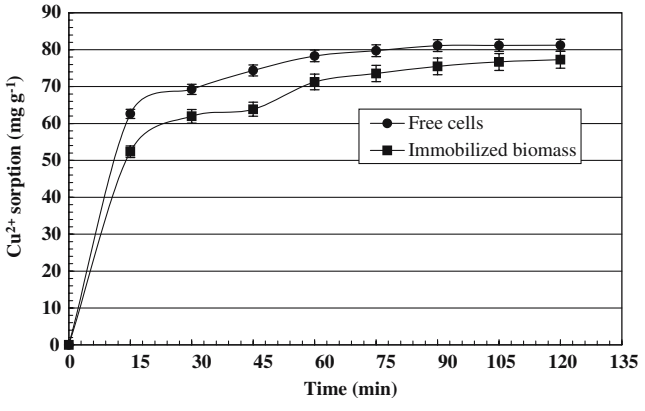

Fig. 4 Cu^{2+} sorption by free and PAG immobilized *S. platensis* biomass. The values for immobilized biomass is the net sorption values obtained after deducting the sorption by biomass-less control cubes

Table 3 Desorption of bound Cu^{2+} by lyophilized PAG immobilized *S. platensis* biomass

Regeneration reagent	Amount of Cu^{2+} desorbed into regeneration reagent (%)	Time required for complete Cu^{2+} desorption (min)
DI water	0.1	90
H_2SO_4 (0.1 mM)	92.3	5
HCl (0.1 mM)	94.3	5
HNO_3 (0.1 mM)	94.6	5
Acetic acid (0.1 mM)	69.1	10
Citric acid (0.1 mM)	79.8	10
NaCl (10 mM)	9.6	20
Na_2CO_3 (10 mM)	85.6	5
$NaHCO_3$ (10 mM)	75.7	5
$CaCl_2$ (10 mM)	70.1	5
$CaCO_3$ (10 mM)	79.1	2
$Ca(NO_3)_2$ (10 mM)	92.1	2
Na_2EDTA (10 mM)	87.3	1

sulfuric acid. Amongst the organic acids, citric acid showed more desorption than the acetic acid. The $Ca(NO_3)_2$ in the category of inorganic salts, showed maximum desorption of Cu^{2+} corresponding to 92.1%, followed by $CaCl_2$ (70.1%) and $CaCO_3$ (79.1%). The selective preference of desorbent by the bound metal can be explained by the stability constants derived from the metal ligand formation [28]. The selective metal desorption has been suggested to result from competition of metal ions and electrostatic non-specific interactions with biomass [29]. The high desorbing efficiency showed by Ca-salts in the present investigation is in agreement with earlier reports on Cd^{2+} and Cu^{2+} elution from marine algae [30] and on Th desorption from *Pseudomonas* biomass [31]. Asthana et al. [32] found Ca $(NO_3)_2$ to be effective in remobilizing Ni^{2+} (84%) from metal loaded biomass in comparison with NaCl. An added advantage is that $CaCl_2$ and Ca $(NO_3)_2$ are very cheap and are mild to the biomass. This makes large-scale operations cost-effective with non-destructive desorption of Cu^{2+} by the *S. platensis* biomass. Our early experiments involving the effect of photosynthetic light and temperature on the metal uptake process showed that the major part of metal uptake is a passive, metabolic energy-independent phenomenon. A recovery of 70–93% of the biomass-bound metal by these desorbents, further confirms our view of non-specific binding at the cell wall. Although the mineral acids showed higher desorption efficiency, they are not preferred because of their damaging effects on biomass. Thus $Ca(NO_3)_2$ was preferred as the suitable desorbent with 92.1% efficiency due to its mild effect on biomass which can be used in repeated cycles.

Study with transmission electron microscopy

Although several metals are required as micronutrient for the growth and metabolism, microorganisms show

cation uptake often at concentrations high enough to be detrimental to them. Thus microbes have developed mechanisms that restrict intracellular metals in selected cell sectors and/or convert them to a more innocuous form such as insoluble phosphide, sulphide, carbide or hydroxide deposits [33]. In an effort to locate the sequestered Cu^{2+}, thin sections of Cu^{2+} equilibrated living cells of *S. platensis* biomass were examined by transmission electron microscopy. The *S. platensis* cells exposed to Cu^{2+} were compared with untreated control cells. As shown by the electron micrographs (Fig. 5), the cell walls of cyanobacterial cells were the main site for the Cu^{2+} deposition. Metal less control cells exhibited a homogenous cytoplasm with a few small electron dense bodies surrounded by the thinner cell envelope where as metal loaded cells showed a distinct, electron-opaque, ring like area at or near the cell periphery indicating the accumulation of Cu^{2+}. Such localized cation deposition on the Gram-negative cell envelope has been attributed to the anionic character of cell wall and membrane components [34]. These observations on Cu^{2+} deposition throughout the cell boundary is also similar to the earlier study, showing preferential sequestration of intracellular lanthanum, silver or cadmium in and around the cell periphery, and leaving a small fraction for the cytoplasm in *P. aeruginosa* [35]. Kuyucak and Volesky [36] also reported similar observations with marine algae *A. nodosum* for Co^{2+} biosorption involving carboxyl, sulfydryl, phosphate, hydroxyl, amino, and amide present in proteins and polysaccharides present in the cell membrane.

Study with infrared spectroscopy

The functional groups involved in Cu^{2+} accumulation by the *S. platensis* was elucidated using IR Spectra obtained at 4000–600 cm^{-1} range (Fig. 5). The IR spectrum showed several peaks reflecting complex nature of the *S. platensis* biomass. The native biomass, comprising different functional groups which regulate the possible cell–cation interactions like H-bonding, complexation, etc. selectively gives following information on accumulation of Cu^{2+}. The native biomass exhibited characteristic absorption at 3500–3000 and 1200–900 cm^{-1}. The first peak lied in a spectrum region occupied by a strong bond (3600–3000 cm^{-1}). The sharp peaks at 3000–2800, 1650, 1600, 1550, and 1400 cm^{-1} were also seen in the spectrum. The IR spectrum of metal treated cells indicated no such shifts or change in any of the characteristic absorbance bands exhibited by the native biomass. The only discernible changes observed were in the length, width, and intensities of the peaks. Especially, there was a 40–45% reduction in peak lengths that belong to the 1650 and 1020 cm^{-1} bands (Fig. 6). A major change in sharpness and intensity of the peak found in the 1650 cm^{-1} band. There was minor change in peak intensity of band related to 1550 cm^{-1}. The IR analysis was made without using a drier box therefore the presence of intense OH peaks in the spectrum could be due to the great hydroscopicity of biomass and the presence of hydroxylic groups in the lyophilyzed biomass. The sharp peak at 3000–2800 cm^{-1} is attributed to the C–H stretching that is characteristic of biological samples. For the native biomass other sharp peaks at 1650 and 1550 cm^{-1} are attribute to the presence of amides-I and -II bands, respectively, (for cellular proteins), followed by carboxylic bands at 1600 and 1400 cm^{-1}. The absorption under 1300 cm^{-1} is characteristic peak of phospate-carrying components, oligo- and poly-saccharides of the cell wall. The weak absorption peaks 720 and 790 cm^{-1} may be attributed to the glycoside bonds in the polysaccharide structure of the biomass. These findings are in conformity with earlier studies on cobalt sorption by an alga [36]. The changes in above-mentioned IR spectrum of metal treated cells indicate the possible involvement of amide, amino, and carboxyl groups in Cu^{2+} accumulation. These changes in peak intensities must be interpreted as changes in concentrations rather than structural changes

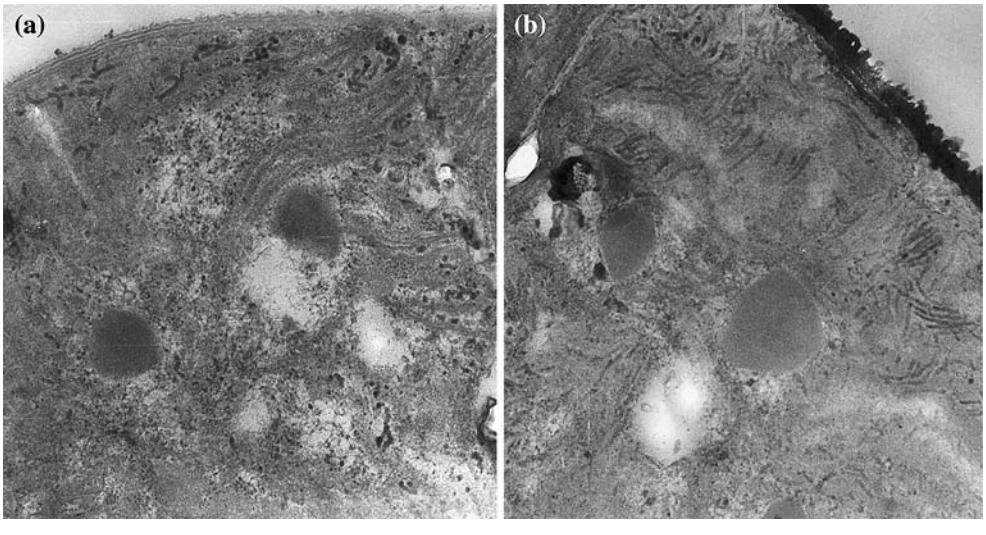

Fig. 5 Transmission electron micrographs of *S. platensis*. **a** Metal untreated control cell and **b** Cell after 2 h of Cu^{2+} (600 mg l^{-1}) treatment; Magnification × 10 000

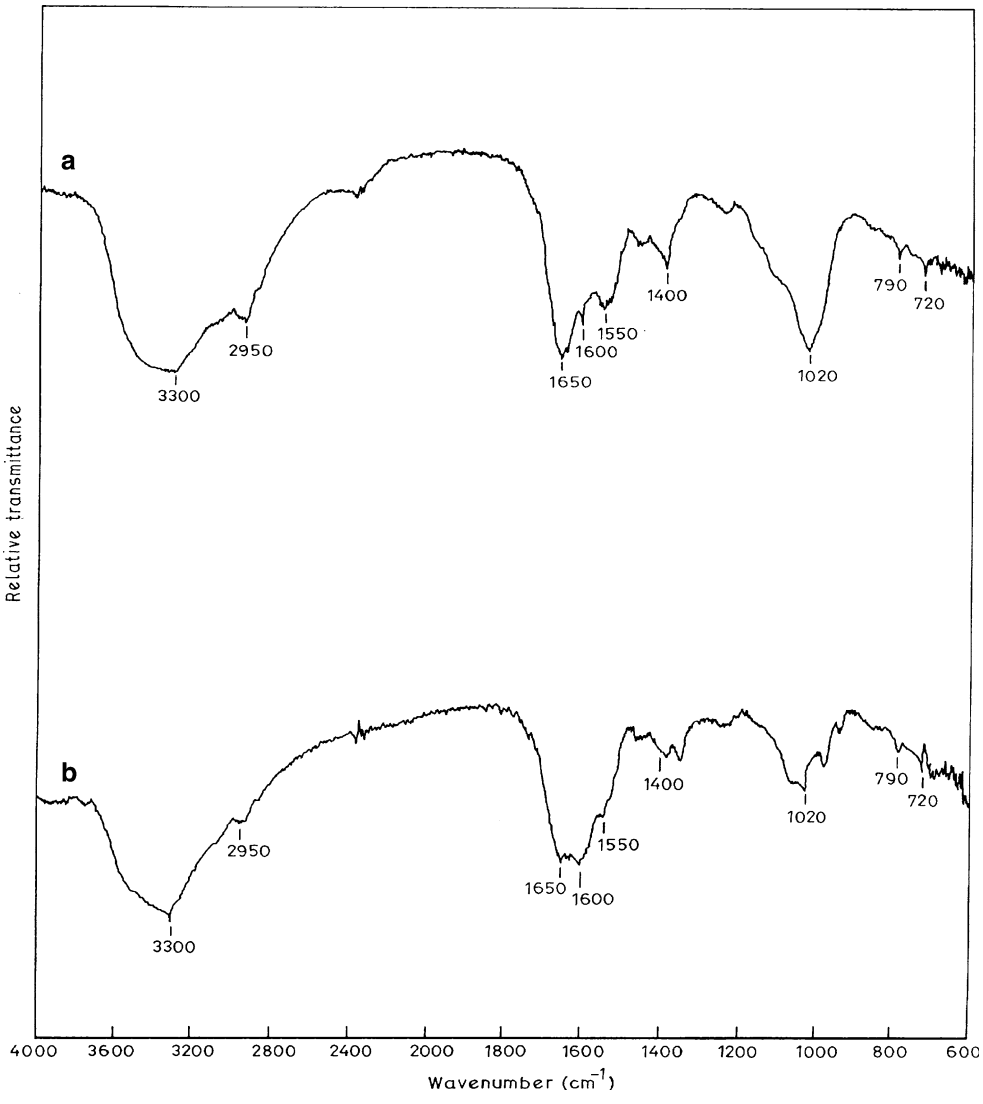

Fig. 6 Infrared spectra of lyophilized *S. platensis* biomass. **a** Metal untreated biomass and **b** Biomass after 2 h of Cu^{2+} (600 mg l^{-1}) treatment

as described by Kuyucak and Volesky [36] for cobalt biosorption on to *Ascophyllum nodosum*.

Column runs

The efficiency of *Spi*SORB was examined under a columnar reactor condition having Cu^{2+} alone or Cu^{2+} in combination with Zn^{2+} (a combination prevalent in electroplating industrial effluent) at various flow rates. The breakthrough curves for pure Cu^{2+} solution (0.1 mg l^{-1}) pumped at the flow rate of 2 ml min^{-1}, showed that the Cu^{2+} appeared in the reactor effluent (breakthrough) after pumping 32.4 l of solution. The amount of Cu^{2+} in the reactor effluent showed a steady increase with time in the reactor attaining a maximum value at 41.6 l of influent volume suggesting saturation of the biomass. A doubling of flow rate (4 ml min^{-1}) showed an early appearance of breakthrough at 27.3 l and saturation after 39.5 l of pumping. The breakthrough and saturation volume decreased with increasing flow rate (Fig. 7a). The continuous fixed bed adsorption of Cu^{2+} by the *Spi*SORB column containing 2.0 g of *S. platensis* is much greater than the previously examined epichlorohydrin-immobilized *Azolla* system, which showed a breakthrough at 12 l of influent pumping [37], against ca. 32 l shown in the present study.

The recovery of metal from Cu^{2+} loaded *Spi*SORB biomass was studied by pumping 10 mM of Ca $(NO_3)_2$ (as optimized for batch studies) in to the column at varying flow rate (2–8 ml min^{-1}). A 325 ml of desorbent was required for complete desorption of Cu^{2+} at the flow rate of 2 ml min^{-1}, the desorbent requirement decreased with increasing flow rate of the desorbent (Fig. 7b). The Cu^{2+} sorption by the *Spi*SORB column from the solution supplemented with Zn^{2+} (2.2 mg l^{-1}) under the similar conditions showed the early appearance of breakthrough for all the flow rates studied (Fig. 8a). The Cu^{2+} desorption pattern from such metal loaded *Spi*SORB biomass also showed the similar

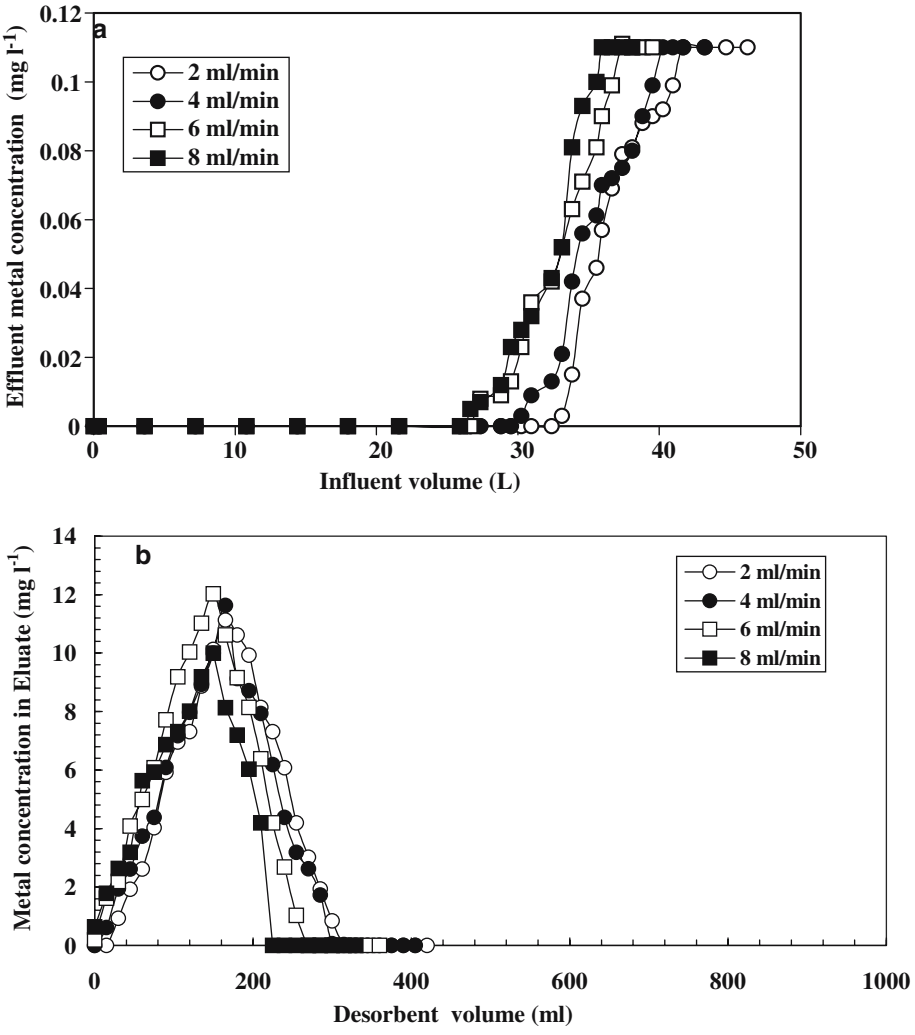

Fig. 7 **a** Experimental breakthrough of Cu^{2+} by PAG immobilized *S. platensis* biomass in Cu^{2+} solution using packed bed column. Metal solution having 0.1 mg l^{-1} of Cu^{2+} was pumped and samples were collected at regular intervals from the outlet as reactor effluent for residual metal estimation. The pH of influent was 6.0, **b** Desorption of Cu^{2+} from PAG immobilized *S. platensis* biomass in packed bed column. 10 mM Ca $(NO_3)_2$ was used as desorbent. The samples were regularly collected from the outlet of the column and assayed for residual metal. The pH of desorbent was 6.0 and pH of metal rich nitrate solution was 5.8

pattern described earlier with the enhanced requirement of the desorbent under bimetallic combination (Fig. 8b). Such results are discussed in terms of volume reduction (a ratio of influent volume for saturation to the volume of desorbent required for complete desorption) and concentration factor obtained after one cycle of sorption and desorption (Table 4). Both these parameters were found to increase with decrease in residence time (corresponding to the increasing flow rate). A maximum of 143-fold volume reduction was observed at the residence time of 4.6 min (flow rate $=6$ ml min^{-1}) for Cu^{2+} alone, which was much higher than the 55-fold reduction obtained in epichlorohydrin-immobilized *Azolla* biomass [37]. The same residence time showed a 109-fold increase in concentration factor, which is higher than 75-fold concentration factor reported with flow rate of 1 ml min^{-1} for *Nostoc*-biosorbent [25]. The presence of Zn^{2+} (2.2 mg l^{-1}) under bi metallic combination with Cu^{2+} (0.1 mg l^{-1}) decreased the efficiency of the biomass. This is reflected by 35% inhibition of volume reduction factor and 8% inhibition in concentration factor at 4.6 min residence time (6 ml min^{-1} of flow rate). The increase or decrease in residence time was found to decrease both the volume reduction and concentration factors.

For the efficient use of PAG immobilized *S. platensis* biomass, the multiple cycles of metal sorption and desorption was carried out in the columnar reactor as shown by Tsezos et al. [38] for uranium recovery by immobilized *Rhizopus arrhizus*. The solution having Cu^{2+} (0.1 mg l^{-1}) was pumped through the column for 112 h, as this duration was found to saturate the biomass in the earlier experiment, at the flow rate of 6 ml min^{-1}. For desorption of Cu^{2+} from the metal loaded biomass 1 h flow of Ca $(NO_3)_2$ was used at 6 ml min^{-1} flow rate. The same column was reused for the subsequent cycles of metal sorption and desorption. As shown in Fig. 9, the first seven cycles showed almost similar value of Cu^{2+} sorption and desorption followed by 30% reduction in both the processes in subsequent cycles. This is again reflected in terms of volume reduction and concentration factors obtained for various cycles. This procedure should improve the process economics of a fixed bed biosorption system as observed by Volesky [4]. The PAG cubes work almost at the same efficiency in terms of volume reduction

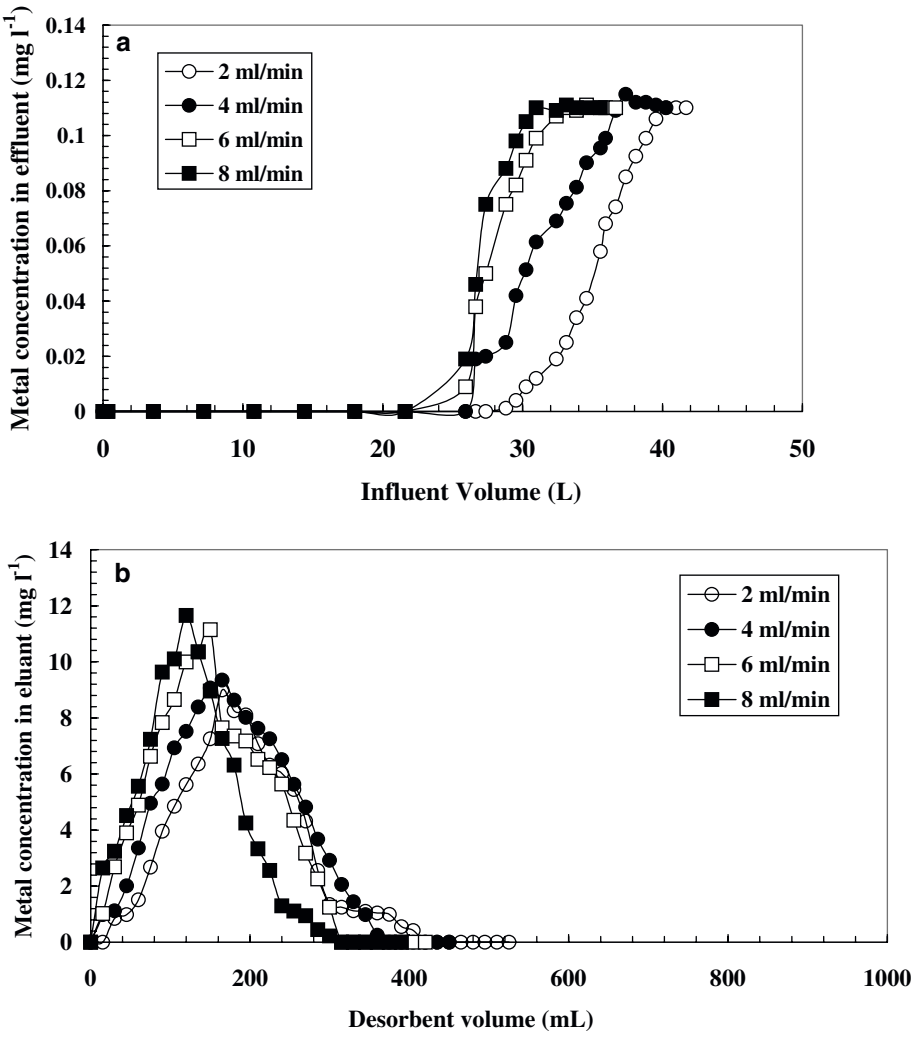

Fig. 8 **a** Experimental breakthrough of Cu^{2+} by PAG immobilized *S. platensis* biomass using surrogate effluent in packed bed column. Metal solution having 0.1 mg l^{-1} of Cu^{2+} and 2.2 mg l^{-1} of Zn^{2+} was pumped and samples were collected at regular intervals from the outlet as reactor effluent for residual metal estimation. The pH of influent was 6.0, **b** Desorption of Cu^{2+} from PAG immobilized *S. platensis* biomass in packed bed column at flow rate of 6 ml^{-1} min. 10 mM Ca $(NO_3)_2$ was used as desorbent. The samples were regularly collected from the outlet of the column and assayed for residual metal. The pH of desorbent was 6.02 and pH of metal rich nitrate solution was 5.9

Table 4 Recovery of Cu^{2+} by the column packed with PAG immobilized *S. platensis* biomass

Residence time (min)	Volume reduction (fold)		Concentration factor (fold)	
	Single metallic system	Double metallic system	Single metallic system	Double metallic system
14	127.1	94.6	101.1	82.4
7	134.3	99.2	105.4	85.5
4.6	143.2	107.1	109.4	101.5
3.5	139.1	105.2	90.5	70.5

and concentration factor for first seven cycles then the efficiency of the columnar reactor goes down suggesting that the same biomass can be reused for seven consecutive cycles of metal sorption and desorption and can treat approximately 270 l of industrial effluent with full efficiency. Having these proven advantages, it is concluded that the *Spi*SORB materials can be considered as good candidates for future investigations in metal removal and recovery processes.

Conclusions

The study of Cu^{2+} biosorption by *S. platensis* in this paper found to be highly feasible for larger scale applications in industry as the loading capacity of the tested biomass found to be reasonably high (ca. 25% dry weight) surpassing the threshold limit of ideal biosorbent. Added interest comes from the fact that the bound metal could be recovered by cheapest reagents like Ca-salts. The quick metal binding and desorption results

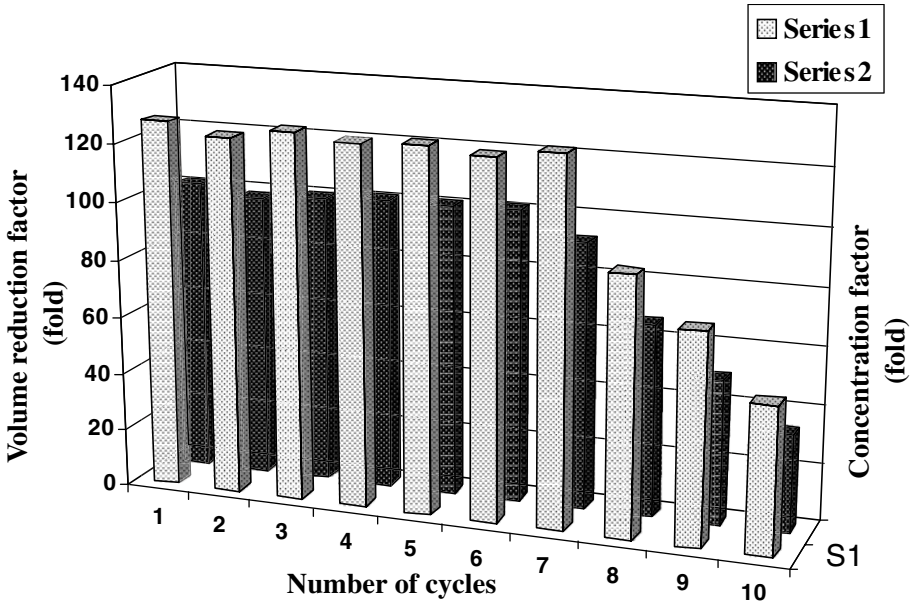

Fig. 9 Performance efficiency of packed bed columnar reactor in terms of volume reduction (*Series 1*) and concentration factor (*Series 2*) over multiple cycles of sorption and desorption

shown by immobilized *S. platensis* upflow packed bed reactor clearly underlines the future potential of using naturally available, abundant microalgae in effluent treatment plants—specially in radionuclide industry because of its lowest liquid to solid ratio obtained in the study.

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