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Remediation of heavy metals using easily cultivable, fast growing, and highly accumulating white rot fungi from hazardous aqueous streams

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ABSTRACT

There has been little investigation into the use of easily cultivable, fast growing, and highly accumulating live white rot fungi (WRF) for the remediation of heavy metal ions contamination. In this regard, the present study was planned to assess the Cu(II), Pb(II), Cr(III), and Cr (VI) remediation potential of live immobilized *Pleutrotus sajor-caju*, *Agaricus bitorquis*, and *Ganoderma lucidum* from aqueous solutions as well as real hazardous effluents. Immobilized *A. bitorquis* had higher heavy metal ions remediation potential as compared to other two strains. The effect of pH, dose, initial metal concentration, time, temperature, etc. on bioremediation potential of WRF were investigated in a batch system. The maximum potential of live immobilized *A. bitorquis* for remediation of Cr(III), Pb(II), Cr(VI), and Cu(II) was 226.6, 208.5, 207.3, and 205.1 mg/g, respectively. Sulfuric acid (0.1 M) was found to be the best desorbing agent. Immobilized *A. bitorquis* remediated heavy metal ions from textile industry wastewater in the following priority order: Cr(III) > Pb(II) > Cr(VI) > Cu(II).

Keywords: Heavy metals; White rot fungi; Bioremediation; Wastewater; Desorption

1. Introduction

Domestic, agricultural, and industrial activities are responsible for the entrance of toxic substances into ecosystem. Behavior, reproduction, survival, growth, and development of the organisms are severely affected when excessive amounts of chemical contaminants enter water [1]. Heavy metals are non-biodegradable unlike organic pollutants. This is the reason they tend to concentrate and accumulate via food chain in living organisms [2]. There is no biological function of heavy metal ions (Cr, Co, Pb, etc.) in living organisms. Metal ions can directly or indirectly damage DNA thereby leading to increased risk of cancer [3]. The conventional methods being currently used for heavy metal removal are membrane processes, electrochemical processes, ion exchange, oxidation-reduction, complexation, electrolysis, reverse osmosis, and precipitation [4,5]. Often these methods involve high operational cost and capital investment. Recently, mycoremediation (fungal bioremediation) has attracted attention of several scientists to overcome heavy metal pollution. However, at extremely high concentration of heavy metal ions, the fungi could be affected by the metal mediated toxic effects. Heavy metal uptake potential of filamentous fungi has been increasingly reported from various parts of the world [6,7]. Wood

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rotting basidiomycetous fungi penetrate wood and lead to easily metabolized, carbohydrate constituents of the complex. Due to versatile machinery of enzymes, the white rot fungi (WRF) are able to attack directly lignin barrier. Efficient branching and filamentous growth of WRF allow for more effective colonization [8–10]. WRF are more suitable for the remediation of heavy metal ions as they can withstand extremely toxic levels of many organic pollutants and can grow in a wide range of temperatures [11,12]. A large number of recalcitrant organic environmental pollutants are decomposed by WRF. Organic pollutant decomposition by WRF was the topic of great concern in past but heavy metal ions uptake and remediation potential of WRF is still needed to be evaluated.

In a previous study, we have found that *Ganoderma lucidum*, *Agaricus bitorquis*, and *Pleutrotus sajor-caju* isolated from Pakistan soils contaminated with heavy metal ions, are easily cultivable, have high metal tolerance and could be exploited for mycoremediation of heavy metal ions from the polluted environment [13]. The results also indicated a direct relationship between level of metal resistance and bioremediation capacity of WRF. Thus, the present research study was undertaken to investigate the bioremediation potential of various WRF for heavy metal ions from synthetic as well as real industrial effluents.

2. Materials and methods

2.1. Reagents

All the chemicals and reagents used in the present study were of analytical grade and mainly purchased from Sigma–Aldrich Chemical Company, USA.

2.2. Microorganism, media, and immobilization

The cultures of white-rot fungi viz. *P. sajor-caju*, *A. bitorquis*, and *G. lucidum* were obtained from Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. The cultures were maintained on potato dextrose agar (PDA) slants. These species were selected for bioremediation purposes on the basis of their metal tolerance ability and visible rapid growth. Seven days old cultures grown on PDA slants were used to prepare spore suspensions for immobilization using Vogel media as described earlier [14]. For the selection of optimum pH for maximum growth of each fungus, the pH of the growth medium varied from 3 to 7 (The results obtained are presented in Table 1). For bioremediation studies, immobilization of basidiospores of each live fungus via entrapment was carried out using the following procedure: two grams of Na-alginate were dissolved in 90 mL of DDW and then mixed with 10 mL of fungal spore suspension having 1×10^9 basidiospore/mL. The whole mixture was dropwise introduced into 0.1 M $CaCl_2$ using a narrow sized burette. The size of beads was found to be 3.55 mm. Calcium chloride solution was constantly stirred to prevent aggregation of Ca-alginate beads. Thus, fungal beads so formed were washed thrice using 300 mL of sterilized DDW. The immobilized fungal spores containing beads were then transferred to a 250-mL capacity Erlenmeyer flask containing 100 mL of growth medium. The beads were subsequently incubated on an orbital shaker set at 150 rpm and 30˚C for 3 days. After completion of incubation period with immobilized fungal mycelia the beads were filtered and washed thrice using DDW. The beads were stored in 50 mM $CaCl_2$ solution maintained at 4˚C until use. Total dry weights of fungal growth on/in beads before and after cell growth were determined by weighing after drying overnight in an electrical oven maintained at 60˚C.

2.3. Bioremediation studies

The uptake of Cu(II), Pb(II), Cr(III), and Cr(VI) ions on the plain alginate beads of immobilized live fungal biomasses from aqueous solutions/wastewater water was investigated both in batch and continuous equilibrium experiments. The effect of pH (4.5), dose (0.05–0.3 g), initial metal concentration (25–1,000 mg/L), time (15–240 min), temperature (20–40˚C), co-metal ions (0–400 mg/L), multi metals (0–400 mg/L), and shaking speed (0–200 rpm) were investigated in a batch system. The pH of the medium was adjusted with 0.1 N HCl or 0.1 NaOH at the beginning of the experiment. After three days of growth of fungal mycelia, the immobilized fungal beads were further incubated for a period of 3 days, thereafter the aqueous phases were separated from the beads and the metal concentration ions in these phases were determined by flame atomic absorption spectrophotometer (Perkin–Elmer AAnalyst 300).

2.4. Desorption

Desorption of metal ions from live WRF was carried out using 0.1 N solution of each of ethylenediaminetetraacetic acid (EDTA), CH_3COOH, H_2SO_4, HCl, and NaOH.

Desorption ratio = amount of desorbed metal ions desorbed/amount of adsorbed metal ions.

Percent desorption values were obtained by multiplying the above ratio by 100.

Table 1
Effect of Vogel's medium pH on growth of WRF

pH	Fungus	Dry wt. (g/L)	Fungus	Dry wt. (g/L)	Fungus	Dry wt. (g/L)
3	*Pleutrotus sajor caju*	0.16	*Agaricus bitorquis*	1.66	*Ganoderma lucidum*	0.24
3.5		0.76		1.70		1.08
4		3.22		2.0		1.28
4.5		1.90		2.20		1.30
5		1.10		2.14		1.34
5.5		1.02		1.94		2.04
6		0.46		1.54		1.44
6.5		0.44		0.76		1.18
7		0.42		0.74		1.14

2.5. Metal ions solution

Stock metal solutions (1,000 mg/L) were prepared by dissolving analytical grade metal nitrates except Cr (VI) for which potassium dichromate was used. Stock solutions (1,000 mg/L) of Cr(III), Cr(VI), Cu(II), and Pb(II) were prepared using 7.695 g of $Cr_3N_3O_9.9H_2O$, 5.6575 g of $K_2Cr_2O_7$, 3.802 g of $CuN_2O_6.{}_3H_2O$, 1.5980 g of PbN_2O_6, 4.9550 g of $Ni(NO_3)_2.6H_2O$, and 4.5490 g of $Zn(NO_3)_2$ in L of DDW. Metal ions solutions of required concentrations were prepared by appropriate dilution of the stock solution (1,000 mg/L) with DDW.

2.6. Determination of metal contents in the metal contaminated solutions

The concentration of metal ions in the aqueous/industrial solutions before and after the equilibrium reached was determined by using a Perkin–Elmer AAnalyst 300 atomic absorption spectrophotometer equipped with an air-acetylene burner, deuterium arc background corrector, and controlled by Intel personal computer. The hollow cathode lamp was operated according to the manual provided by Perkin–Elmer. Polypropylene flasks and glassware were kept immersed in 10% v/v HNO_3 overnight before the analysis procedure. Before use, polypropylene flasks and glassware were rinsed several times with DDW.

2.7. Metal ion uptake capacity

The metal ion uptake by WRF was calculated by the concentration difference method described by Volesky and Holan [15]. The initial metal concentration, C_o (mg/L), and metal concentrations at any time, C_e (mg/L) were determined using AAS analysis. The metal uptake of WRF q (mg/g) was calculated from the mass balance as follows:

$$q = (C_o - C_e)V/1,000 \times W$$

where V = volume of the solution in mL; W = mass of the sorbent in g.

2.8. Statistical analysis

Standard deviation was calculated for three independent determinations for each variable [16]. The level of significance between different observations was determined using *F*-test. The linear regression analysis was carried out to calculate the parameters of Langmuir adsorption isotherm, Freundlich adsorption isotherm, pseudo-first-order kinetic model and pseudo-second-order kinetic model.

3. Results and discussion

3.1. Influence of initial pH

Selection of optimum pH is necessary for maximum metal ion uptake from aqueous solutions [15]. However, for bioremediation studies, selection of optimum pH is required for maximum growth of live fungal cells. The mycelial growth of *P. sajor-caju*, *A. bitorquis*, and *G. lucidum* at first increased with increase in pH, and then decreased after reaching a maximum value. The optimum pH for maximum growth of *P. sajor caju*, *A. bitorquis*, and *G. lucidum* was 4, 4.5, and 5.5, respectively (Table 1).

Immobilized live *P. sajor caju*, *A. bitorquis*, and *G. lucidum* remediated Pb(II) in preference to Cu(II), Cr(III), and Cr(VI). Immobilized live *A. bitorquis* biomass exhibited more metal ion remediation potential than immobilized live *P. sajor caju* and *G. lucidum* (Fig. 1(a)). Due to higher metal ion uptake capacity of

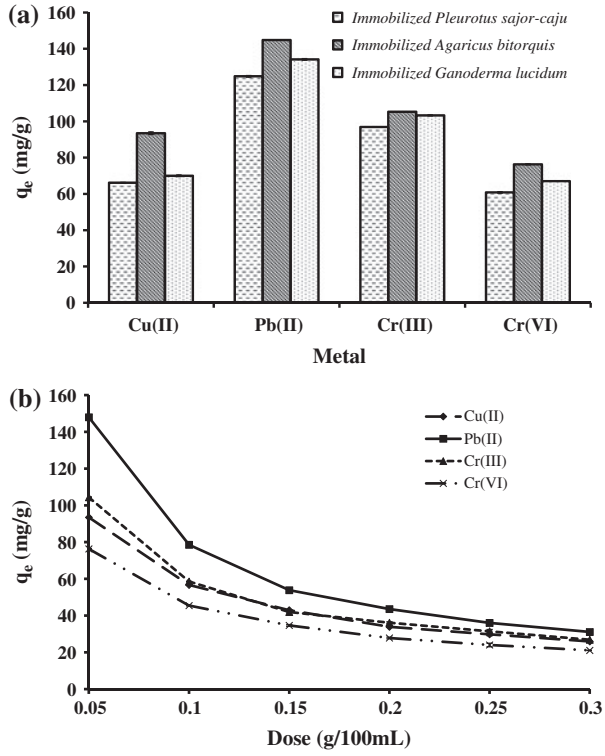

Fig. 1. (a) pH studies for Cu(II), Pb(II), Cr(III), and Cr(VI) uptake by immobilized live fungal biomasses. (b) Effect of immobilized live *A. bitorquis* biomass dose on metal uptake.

immobilized live *A. bitorquis* biomass, it was selected to carry out remaining part of the present study. For remediation of Cu(II), Pb(II), Cr(III), and Cr(VI), the solution pH was set at pH 4.5. This pH was selected to facilitate the growth of immobilized live *A. bitorquis*.

3.2. Effect of biosorbent dose

Biosorbent dose is an important factor for bioremediation studies as it determines the sorbent and sorbate equilibrium of the system. The influence of biosorbent dose on metal ion uptake ability of immobilized live *A. bitorquis* biomass was evaluated by varying dosage from 0.05 to 0.3 g/100 mL at pH 4.5, 120 rpm shaking speed and 30˚C (Fig. 1(b)). Cu(II), Pb(II), Cr(III), and Cr(VI) uptake capacity (q_e) of immobilized live *A. bitorquis* biomass decreased as the dosage of biomass was increased. High biomass doses cause the agglomeration of cells as well as reduce the inter-cellular distance. As a result "screening effect" is produced among cells due to denser layers which lead to "protection" of the sites available for binding metal ions. In simpler words, more metal ions are taken up by biomass when the inter-cellular distance is more as at low biosorbents concentrations. Under such conditions, cells have optimal electrostatic interaction which is a significant factor for metal ions uptake [17].

3.3. Effect of initial metal concentration

The rate of metal ions adsorption/uptake is dependent upon metal ions concentration present in

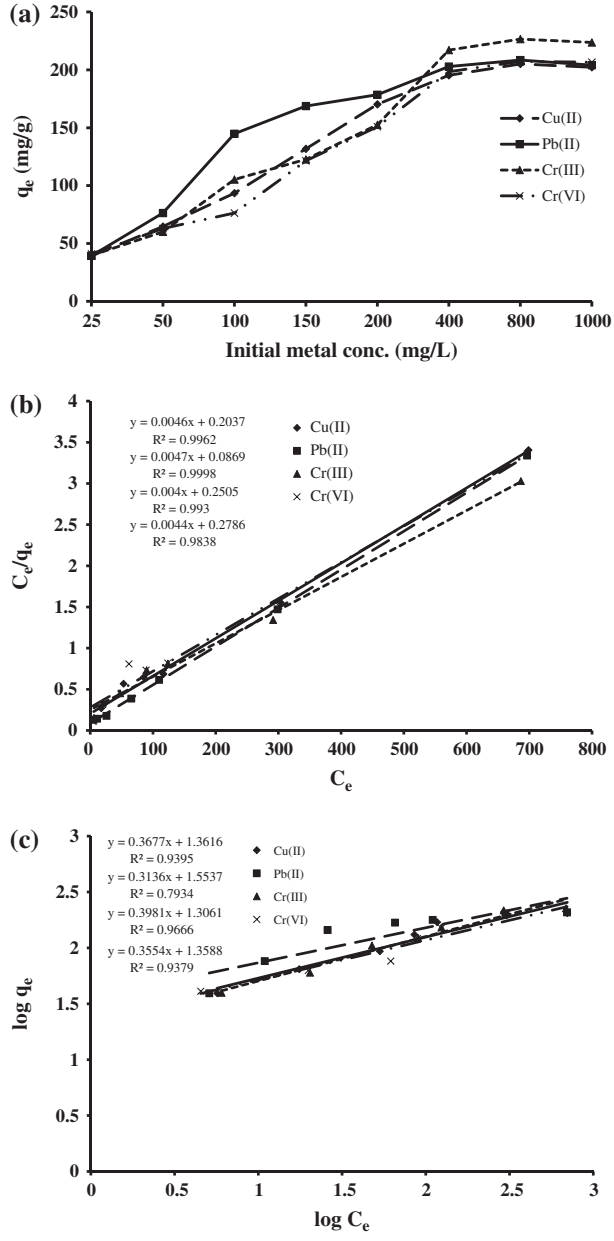

Fig. 2. (a) Effect of initial metal concentration on metal uptake by immobilized live *A. bitorquis* biomass. (b) Langmuir adsorption isotherm for metal uptake by immobilized live *A. bitorquis* biomass. (c) Freundlich adsorption isotherm for metal uptake by immobilized live *A. bitorquis* biomass.

Table 2
Comparison between Langmuir and Freundlich adsorption isotherm parameters for metal uptake by immobilized live *A. bitorquis* biomass

Metal	Langmuir isotherm parameters			Experimental value	Freundlich isotherm parameters			
	q_{max} (mg/g)	K_L (L/mg)	R^2	q_{max} (mg/g)	q_{max} (mg/g)	K (mg/g)	$1/n$	R^2
Cu(II)	217.39	2.26×10^{-2}	0.9962	205.12	126.19	22.30	0.3677	0.9395
Pb(II)	212.76	5.41×10^{-2}	0.9998	208.5	153.66	35.79	0.3136	0.7934
Cr(III)	250	1.60×10^{-2}	0.9930	207.32	133.01	20.23	0.3981	0.9666
Cr(VI)	227.27	1.59×10^{-2}	0.9838	226.62	118.89	22.84	0.3554	0.9379

aqueous system. The effect of initial metal concentration Cu(II), Pb(II), Cr(III), and Cr(VI) ions on the metal uptake capacity (q_e) of immobilized live *A. bitorquis* biomass was evaluated at 25–1,000 mg/L and the results are shown in Fig. 2(a). It is obvious from the results that metal ion uptake capacity of the fungus increased with increase in initial metal ion concentration up to 800 mg/L. A further increase in concentration exhibited a small decrease in metal uptake capacity. Such a reduction in Cu(II), Pb(II), Cr(III), and Cr(VI) uptake capacity (q_e) of immobilized live *A. bitorquis* biomass was due to saturation of adsorption sites as well as reduction in growth of fungal cells. The metal uptake capacity (mg/g) of immobilized live *A. bitorquis* biomass was in the following order: Cu(II) (205.12) > Pb(II) (208.5) > Cr(VI) (207.32) > Cr(III) (226.62). The initial metal concentration data was fitted to Lagmuir and Freundlich sorption isotherm models (Fig. 2(b) and (c)) to describe the nature of metal uptake by immobilized live *A. bitorquis* biomass. A comparison between Langmuir and Freundlich adsorption isotherm parameters for Cu(II), Pb(II), Cr(III), and Cr(VI) ions uptake by immobilized live *A. bitorquis* biomass is presented in Table 2. Estimated q_{max} of Langmuir adsorption isotherm correlated well to the experimental q_{max} values. Secondly, values of correlation coefficient (R^2) were also higher in case of Langmuir adsorption isotherm model than those from Freundlich adsorption isotherm model. Thus, the results suggested that the equilibrium data fitted well to the Langmuir adsorption isotherm as compared to other models. Iqbal and Saeed [18] also reported that entrapment of fungal hyphae in structural fibrous network of papaya wood produced a unique biosorbent for the removal of heavy metals. Moreover, metal ion uptake process was well described by the Langmuir adsorption isotherm model.

3.4. Effect of shaking speed

The metal ions uptake by live biomass was evaluated varying the agitation rate from 0 rpm (without agitation) to 200 rpm, aiming at determining the optimal shaking rate (Fig. 3). It is evident from Fig. 3 that the removal efficiency of all four metal ions increased as agitation speed increased from 0 to 200 rpm. It is obvious that at lower agitation speed, there was insufficient energy for metal ions to permeate the intra particular surface for its uptake by biomass [14].

3.5. Effect of temperature

The effect of temperature on the metal ions bioremediation by immobilized live *A. bitorquis* was

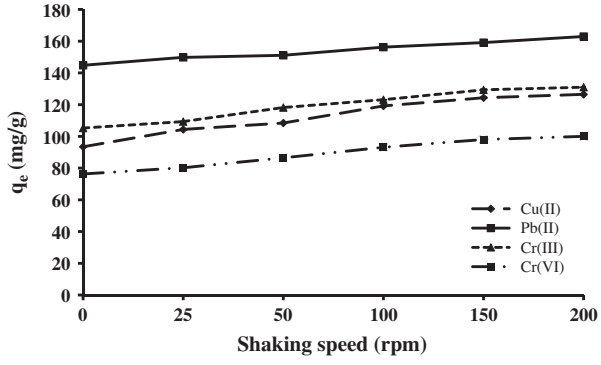

Fig. 3. Effect of shaking speed on Cu(II), Pb(II), Cr(III), and Cr(VI) uptake by immobilized live *A. bitorquis* biomass.

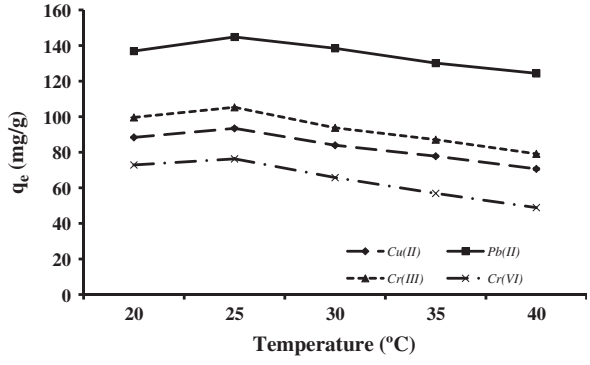

Fig. 4. Effect of temperature on Cu(II), Pb(II), Cr(III), and Cr(VI) uptake by immobilized live *A. bitorquis* biomass.

investigated at five different temperatures (20–40˚C) and the results are depicted in Fig. 4. The result revealed that maximum uptake for metal ions was observed at 25˚C. This might have been occurred due to high production of fungal mycelia at this temperature. At higher temperatures, metal removal decreases due to decrease in growth of fungal cells. The dependency of metal uptake process on solution temperature was observed by Arica and Bayromoglu [19]. They found that Cr(VI) uptake by free and CMC immobilized *Lentinus sajor-caju* mycelia was affected by temperature in the range of 5–40˚C.

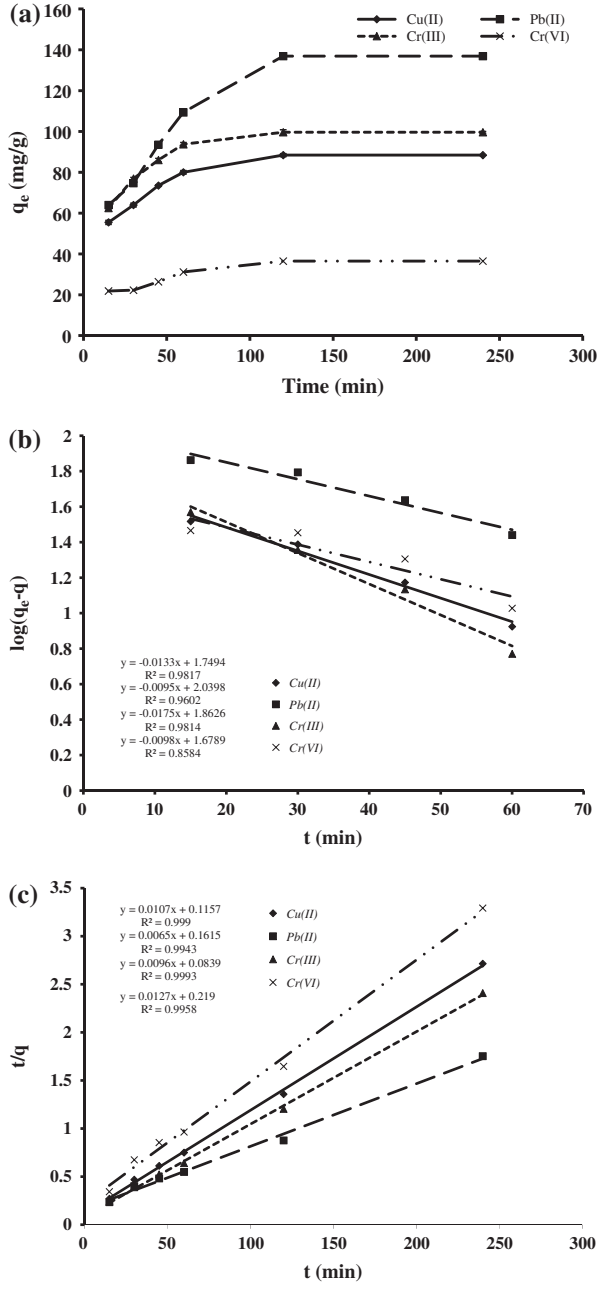

Fig. 5. (a) Effect of time on Cu(II), Pb(II), Cr(III) and Cr(VI) uptake by immobilized live *A. bitorquis* biomass. (b) Pseudo-first-order kinetic model for Cu(II), Pb(II), Cr(III), and Cr(VI) uptake immobilized live *A. bitorquis* biomass. (c) Pseudo-second-order kinetic model for Cu(II), Pb(II), Cr (III) and Cr(VI) uptake immobilized live *A. bitorquis* biomass.

3.6. Effect of time

It was observed that metal uptake by immobilized live *A. bitorquis* fungal biomass increased with time and equilibrium was attained after 120 min (Fig. 5(a)). The time required to attain this state of equilibrium was termed as the equilibrium time and the amount of ions adsorbed at this equilibrium time reflected the maximum biosorption capacity of the biomass under the particular conditions. Tables 3 and 4 represent the kinetic data of studies carried out on synthetic wastewater in batch and continuous modes, respectively. As the volume of industrial effluents is usually large, it is good to carry out laboratory studies in continuous mode to get an idea regarding real time adsorption. The result of kinetic studies carried out for industrial effluents is presented in Table 5.

The results obtained for contact time experiments for synthetic wastewater in batch mode were fitted to pseudo-first-order and pseudo-second-order kinetic models (Fig. 5(b) and (c)) [15]. High value of correlation coefficient (R^2) and a close agreement between estimated and experimental q values suggested the fitting of pseudo-second-order kinetic model (Table 3) to remediation data of Cu(II), Pb(II), Cr(III), and Cr(VI). Kinetic modeling of synthetic and real wastewater in continuous mode is presented in sections 3.8 and 3.10, respectively.

3.7. Effect of co-metal ions

The data depicted in Fig. 6(a)–(h) were obtained for co-cation uptake by immobilized live *A. bitorquis* biomass. There was reduction in metal ions uptake in the presence Na, Ca^{2+}, and Al^{3+} ions. The extent of inhibition in metal ions uptake in the presence of co-metal ions was in following order $Al^{3+} > Ca^{2+} > Na$. In the present study, a co-cation concentration dependent direct competition for binding sites was observed. These results supported the hypothesis of the presence of a multiplicity of binding sites in the biomass [20].

The competitive bioremediation of Cu(II), Pb(II), Cr(III), and Cr(VI) was studied by fixing the concentration of metal under study at 100 mg/L, while varying the concentration of other ions from 0 to

Table 3
A comparison between pseudo-first and second-order kinetic models for Cu(II), Pb(II), Cr(III) and Cr(VI) uptake uptake from synthetic wastewater in batch mode by immobilized live *A. bitorquis* biomass

Metal	Pseudo-first-order kinetic model			Experimental value	Pseudo-second-order kinetic model		
	q_e (mg/g)	$K_{1,ads}$ (min^{-1})	R^2	q_e (mg/g)	q_e (mg/g)	$K_{2,ads}$ (g/mg min)	R^2
Cu(II)	116.30	6.47×10^{-2}	0.9545	93.46	97.08	1.33×10^{-3}	0.9982
Pb(II)	145.11	3.61×10^{-2}	0.9894	144.84	158.73	3.58×10^{-4}	0.9968
Cr(III)	74.63	4.90×10^{-2}	0.9859	105.28	108.69	1.48×10^{-3}	0.9994
Cr(VI)	62.37	3.20×10^{-2}	0.9512	76.30	81.97	8.06×10^{-4}	0.9974

Table 4
A comparison between pseudo-first and second-order kinetic models for Cu(II), Pb(II), Cr(III) and Cr(VI) uptake from synthetic wastewater in continuous mode by immobilized live *A. bitorquis* biomass

Metal	Pseudo-first-order kinetic model			Experimental value	Pseudo-second-order kinetic model		
	q_e (mg/g)	$K_{1,ads}$ (min^{-1})	R^2	q_e (mg/g)	q_e (mg/g)	$K_{2,ads}$ (g/mg min)	R^2
Cu(II)	32.13	2.57×10^{-2}	0.9905	83.36	82.21	1.71×10^{-3}	0.9997
Pb(II)	147.77	4.51×10^{-2}	0.9841	136.90	147.06	5.22×10^{-4}	0.9975
Cr(III)	74.13	3.31×10^{-2}	0.8221	98.0	104.17	7.44×10^{-4}	0.9973
Cr(VI)	41.46	6.15×10^{-2}	0.9211	68.18	69.44	4.42×10^{-4}	0.9998

Table 5
A comparison between pseudo-first and second-order kinetic models for Cu(II), Pb(II), Cr(III) and Cr(VI) uptake from real textile effluent in continuous mode by immobilized live *A. bitorquis* biomass

Metal	Pseudo-first-order kinetic model			Experimental value	Pseudo-second-order kinetic model		
	q_e (mg/g)	$K_{1,ads}$ (min^{-1})	R^2	q_e (mg/g)	q_e (mg/g)	$K_{2,ads}$ (g/mg min)	R^2
Cu(II)	31.08	1.47×10^{-2}	0.9548	26.37	156.25	2.74×10^{-5}	0.6049
Pb(II)	48.08	2.14×10^{-2}	0.9113	41.49	107.52	5.14×10^{-5}	0.9564
Cr(III)	67.17	2.97×10^{-2}	0.9985	66.08	77.51	9.10×10^{-5}	0.9944
Cr(VI)	46.39	2.48×10^{-2}	0.9705	38.92	92.59	6.99×10^{-5}	0.9713

400 mg/L. It was not possible to study the effect of Cr(III) on Cr(VI) uptake or vice versa due to formation of metal complex. The results showed that the equilibrium uptakes of each metal ion were reduced by the presence of other metals in the mixture. Increasing in concentrations metal ions caused the decreasing individual adsorption yields of metal under study. As a result, the effect of the mixture was found less than that of each of the individual effects of the constituents in the mixture so the interaction between metal ions could be assumed to be antagonistic. The most logical reason for the antagonistic action could be explained as being the competition for adsorption sites on the cells in the case of cadmium(II) and nickel(II) [21]. Similar observations were reported in the studies on metal sorption from multi-metal solutions with a waste biomass of *Streptomyces noursei* [22]. The sorption preference for Pb^{2+} could be due to its higher atomic weight.

3.8. Column study

A Pyrex glass column having 75 cm height and 1.8 cm internal diameter was packed with 10 g of immobilized live *A. bitorquis* biomass. The bed depth and bed volume so obtained were 53 cm (approximately) and 120 mL, respectively. To assure even circulation of metal solution and also to prevent the fungal beads from floating 1 cm height on both ends

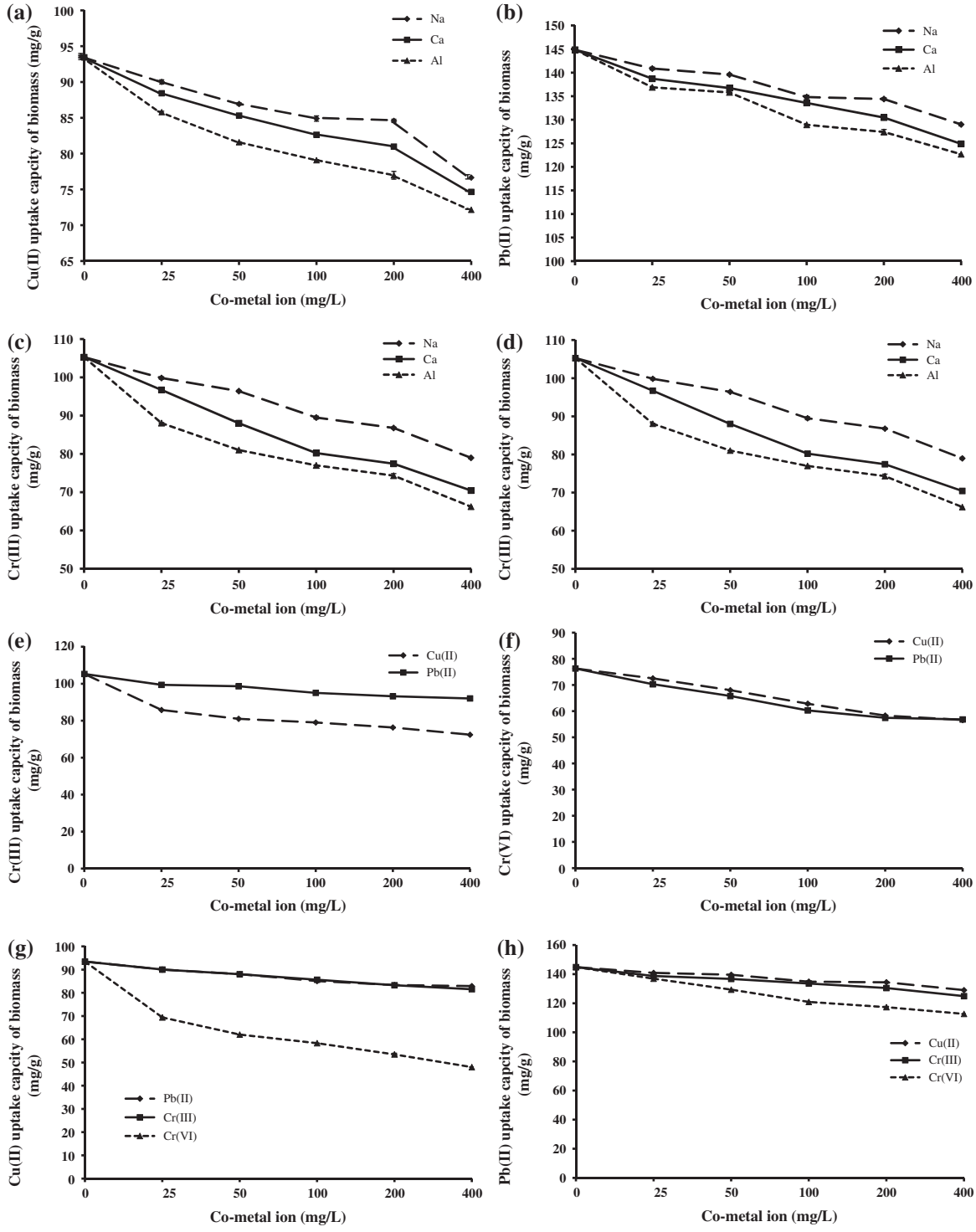

Fig. 6. (a) Effect of co-metal ions on Cu(II) uptake by immobilized live *A. bitorquis* biomass. (b) Effect of co-metal ions on Pb(II) uptake by immobilized live *A. bitorquis* biomass. (c) Effect of co-metal ions on Cr(III) uptake by immobilized live *A. bitorquis* biomass. (d) Effect of co-metal ions on Cr(VI) uptake by immobilized live *A. bitorquis* biomass. (e) Effect of Cu(II) and Pb(II) concentration on Cr(III) uptake by immobilized live *A. bitorquis* biomass. (f) Effect of Cu(II) and Pb(II) concentration on Cr(VI) uptake by immobilized live *A. bitorquis* biomass. (g) Effect of Pb(II), Cr(III), and Cr(VI) concentration on Cu(II) uptake by immobilized live *A. bitorquis* biomass. (h) Effect of Cu(II), Cr(III), and Cr(VI) concentration on Pb(II) uptake by immobilized live *A. bitorquis* biomass.

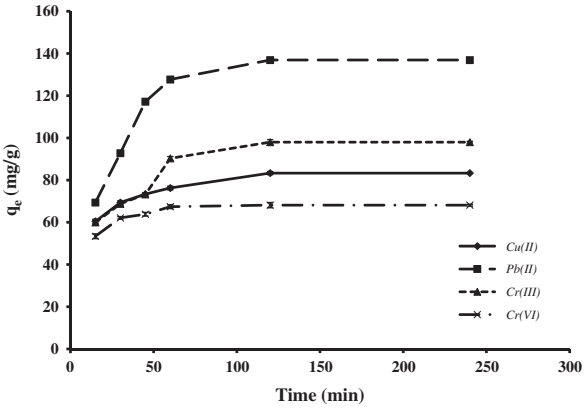

Fig. 7. Column studies immobilized live *A. bitorquis* biomass.

of the glass column was filled with glass beads. At the bottom of the column glass, wool was placed between the glass beads and the biomass beads. The bottom of the column was sealed using a rubber stopper with a single bore. The connection in the column setup was made using Tygon tubing. The down flow mode was selected to operate the column. The flow rate of continuous aqueous phase was 2.5 mL/min which was maintained using a peristaltic pump. The initial concentration of metal solution was 100 mg/L and solution pH was maintained at 4.5 to facilitate the growth of immobilized live *A. bitorquis* biomass. The concentration of metal ions in the eluent phase was determined after predetermined time intervals. The results obtained clearly demonstrated that metal removal was higher in batch setup in comparison with continuous setup (Fig. 7). Mungasavalli et al. [23] studied removal of chromium from aqueous solution using *Aspergillus niger* in batch and continuous mode. The results indicated that maximum removal of chromium was observed in batch mode. A comparison between pseudo-first-order and pseudo-second-order kinetic models is tabulated in Table 4. A careful examination of the parameters obtained from both models clearly demonstrates that the best fit model to the results obtained was pseudo-second-order kinetic model.

3.9. Desorption

Metal remediation studies are incomplete unless biomass regeneration studies accompany them. Five desorbing agents including EDTA, hydrochloric acid, sulfuric acid, nitric acid, and sodium hydroxide were used to regenerate immobilized live *A. bitorquis* biomass (Fig. 8). The amount of metal eluted was compared to initial amount of metal present in biomass before the start of experiment to calculate percent of metal desorbed. The effectiveness of desorbing agents used in the present study was in the following order: sulfuric acid < hydrocholoric acid < sodium hydroxide < acetic acid < EDTA. Almost 81.46, 90.35, 74.35, and 69.81% of Cu(II), Pb(II), Cr(III), and Cr(VI) were recovered from immobilized live *A. bitorquis* biomass using sulfuric acid as desorbing agent. Unfortunately, it was not possible to reuse the live biomass with the same metal uptake capacity in a new sorption–desorption cycle due to the death of growing cells in the presence of corrosive reagents such as acetic acid, sulfuric acid, hydrochloric acid, and sodium hydroxide. The possibility of reusing the live biomass after using EDTA as desorbing agent was possible but it was at the expense of losing almost 30% metal uptake capacity. However, EDTA was used as a regenerating agent without any further loss in metal uptake capacity. The reduction in Cu(II), Pb(II), Cr(III), and Cr(VI) uptake by regenerated immobilized live *A. bitorquis*

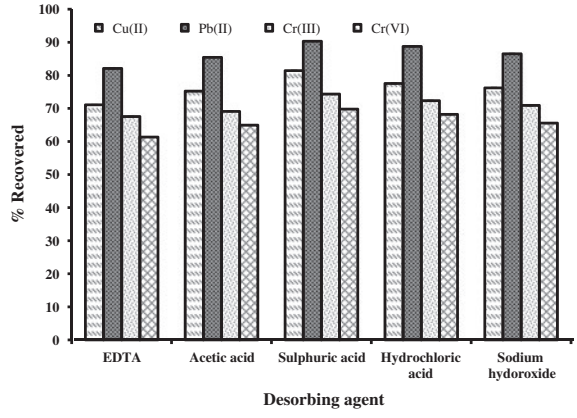

Fig. 8. Desorption of metal ions from immobilized live *A. bitorquis* biomass.

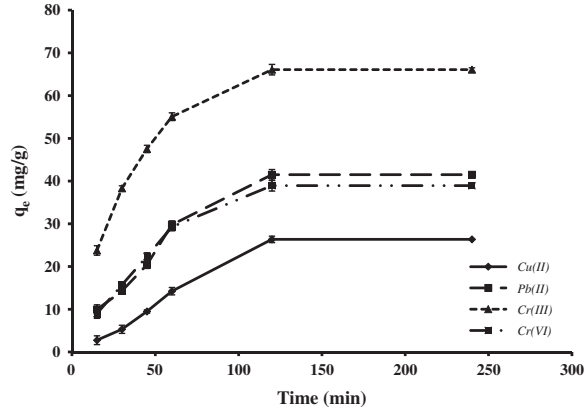

Fig. 9. Industrial effluent immobilized live *A. bitorquis* biomass.

Table 6
A comparison among metal uptake capacities (mg/g) of various biomaterials

Biomass	Metal studied	Uptake capacity (mg/g)	Reference
Agaricus bitorquis (living cells)	Cu(II)	205.12	The present study
	Pb(II)	208.5	
	Cr(III)	207.32	
	Cr(VI)	226.62	
Fusarium oxisporum	Cu (II)	7.52	[30]
Rhizopus (RSH-9)	Cr	9.50	[31]
Aspergillus niger	Cu (II)	20.91	[32]
Aspergillus niger	Pb (II)	54.04	
Punica granatum	Pb(II)	68.74	[33]
Carum copticum	Cr(VI)	3.813	[34]
Agaricus bitorquis (dead cells)	Cr(III)	152.74	[35]
	Cr(VI)	127.92	
Pleutrotus sajor-caju (dead cells)	Cr(III)	141.88	
	Cr(VI)	122.36	
Ganoderma lucidum (dead cells)	Cr(III)	149.58	
	Cr(VI)	127.28	

biomass was associated with interactions between metal-sorbing sites present on biomass and the complexing agent. This might be due to irreversible blocking of adsorption sites by metal already adsorbed onto biomass cells [24].

3.10. Industrial effluent

Wastewater sample from the textile industry was subjected to a continuous type bioremediation experiment using same column setup as described in section 3.6. The effect of contact time on Cu(II), Pb(II), Cr(III), and Cr(VI) uptake by immobilized live *A. bitorquis* biomass was studied under predefined time interval (Fig. 9). For industrial-scale application of bioremediation phenomenon, selection of optimum operating conditions in continuous phase is necessary [25,26]. The pH of solution was maintained up to 4.5. The metal uptake was very rapid and equilibrium reached after 120 min. The metal uptake in the case of industrial wastewater was comparatively lower in comparison with that in synthetic wastewater. This might be due to the presence of large number of contaminants in the textile wastewater which might have competed with metal ions for adsorption sites [27]. Similarly, Kumar et al. [28] reported that removal of Cr(VI) was less from the electroplating wastewater using fungal biomass as compared to synthetic wastewater.

Two most extensively used mathematical models viz. pseudo-first-order and pseudo-second-order kinetic models were used to describe Cu(II), Pb(II), Cr(III), and Cr(VI) uptake by immobilized live *A. bitorquis* biomass. A kinetic model was finally selected which precisely fitted to the data as well as had high value of correlation coefficient (R^2). Pseudo-first-order kinetic model exhibited best fit in case of continuous metal uptake from the textile industry wastewater (Table 5). The fitting of pseudo-first-order kinetic model to fungal data was also reported by Bayramoglu et al. [29] in a bioremediation study. The fitting of pseudo-first-order model to data of industrial wastewater suggested that there was a different metal uptake process in case of industrial effluents.

4. Conclusions

The present study demonstrated the relatively high binding capacity of immobilized live *A. bitorquis* biomass for heavy metal ions in comparison to previously reported biosorbents (Table 6). Heavy metal ions like Cu(II), Pb(II), Cr(III), and Cr(VI) are remediated from the synthetic as well as real effluents. The pH, initial metal ion concentration, and contact time significantly affected the bioremediation potential of *A. bitorquis*.

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