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Biosorption of Cr(III) and Cr(VI) by Newly Isolated White Rot Fungi: Batch and Column Studies

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The present study was planned to evaluate the heavy metals uptake potential of newly isolated white rot fungi from metals contaminated sites. Three white rot fungi *viz.*, *Pleutrotus sajor-cajor, Ganoderma lucidum* and *Agaricus bitorquis* were selected for biosorption of Cr(III) and Cr(VI) studies. The optimum pH for maximum uptake of Cr(III) and Cr(VI) by native/immobilized *Pleurotus sajor-caju*, *Agaricus bitorquis* and *Ganoderma lucidum* was found to be 5 and 2, respectively. Increase in biomass concentration in a fixed volume of solution (100 mL) at constant pH 5, initial metal concentration (100 mg/L) and temperature (30 ºC) reduced the metal sorption capacity of biomass. Biosorption of both metal ions increased with concentration from 25 to 200 mg/L. Langmuir adsorption isotherm model and pseudo second order kinetic model fitted well to metal biosorption data. Equilibrium for metal uptake was reached within 2 h of contact. Metal uptake process was found dependent on temperature. The pseudo second order kinetic model fitted well to experimental data of column study as it had higher value of correlation coefficient (R^2). Sulphuric acid was found as the best eluent for metal recovery from dead fungal biomass.

Key Words: White rot fungi, Chromium, Biosorption, Column studies, Immobilization.

INTRODUCTION

Domestic, agricultural and industrial activities are responsible for the entrance of toxic substances into natural water. Behaviour, reproduction, survival, growth and development of the organisms severely effected when excessive amounts of chemical contaminants enter into water bodies[1-3]. Heavy metal ions are non-biodegradable unlike organic pollutants. Although a number of methods available for heavy metal removal but adsorption is one of the most applicable methods[4-7]. There is no biological function of heavy metal ions (Cr, Co, Pb *etc*.) in living organisms. Copper, zinc and nickel are essential at low concentrations but at high levels these are toxic. Metal ions can directly and indirectly damage DNA and that means an increased risk of cancer. This is known as genotoxicity. Heavy metal ions also cause immuno-toxicity. Extremely toxic and carcinogenic effects of soluble metals on biological systems are due to strong oxidizing power of heavy metals[8-10].

The conventional methods (membrane processes, electrochemical processes, ion exchange, oxidation-reduction, complexation, electrolysis, reverse osmosis and precipitation) being used for heavy metal pollution removal, involve high operational cost and high capital investment. The generation of secondary wastes (pollutants) present treatment problems associated with these methods. Toxic heavy metals from industrial effluents can be sequestered by inexpensive dead biomass. This method is referred as biosorption. Abundant dead plant, moss, algae, bacteria and fungi were used as biosorbents in the past. But the scientists are still in search of new abundant and inexpensive biomaterials with high metal uptake capacity. Biosorption has several advantages like minimization of waste sludge, high efficiency, low cost, no additional nutrient requirement, biosorbent regeneration and metal recovery over conventional wastewater treatment methods[9,11-13].

Biosorption mechanism is complex mainly comprised of adsorption, chelation, ion exchange, intra and interfibrilliar entrapment, diffusion, *etc*. Two phases are involved in biosorption process. One is solid phase (biosorbent) other is liquid phase (wastewater). On the basis of affinity metal/s present in wastewater is/are bound and attracted by various mechanisms by biosorbent. Till the establishment of equilibrium between the unbound and bound sorbate species, the biosorption process remains continues. The biosorption process is strongly dependent on physio-chemical parameters such as pH, biosorbent dose, biosorbent particle size, ionic strength, concentration of co-metal ions, contact time and temperature. A fungus that is capable of degradation of lignin and lignin like compounds is termed as white rot fungi. White rot fungi are basidiomycetes.

A careful survey of the literature indicates the lack of biosorption data on Cr(III) and Cr(VI) using white rot fungi. Eleven white rot fungi (*viz.*, *Lintus edodes, Podaxis pistillaris, Pleutrotus ostreatus, Pleutrotus ostreatus* (small stem), *Pleutrotus sajor-cajor, Genoderma lucidum, Agaricus bisprous, Agaricus bitorquis* (J_{77}), *Agaricus bitorquis* (A_{61}), *Agaricus bitorquis* (A_{65}) and *Agaricus bitorquis* (A_{67})) were isolated from those Pakistani metal contaminated agricultural soils which were receiving long-term (> 50 years) application of industrial and municipal wastewaters. Such a long term exposure of fungi to pollutant can result into physiological adaptation to contaminated environment as well as increase in metal sorption capacity. The present research study was carried out to determine metal uptake capacity of various white rot fungi native to Pakistan.

EXPERIMENTAL

Analytical grade reagents mainly purchased from Sigma-Aldrich Chemical Company were used in the present study.

Microorganism collection, identification and preparation: White rot fungi used in the present study were isolated from agricultural soils irrigated with industrial/municipal wastewater. Multi metal tolerant white rot fungi were collected in potato dextrose agar (PDA) using the standard spread plate method. To a liter of potato dextrose agar 0.5 g of streptomycin sulfate was added for bacterial growth inhibition. For tentative identification of pure fungal cultures at the genus level macroscopic (*viz.*, shape, colour, texture, colonial morphology, appearance of colony and diameter) and microscopic (*viz.*, shape, structure of conidia, presence of specific reproductive structures and presence of sterile mycelium) characteristic were identified in Mushroom Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. For initial screening of white rot fungi, all collected fungi *viz.*, *Lintus edodes, Podaxis pistillaris, Pleutrotus ostreatus, Pleutrotus ostreatus* (small stem), *Agaricus bisprous, Ganoderma lucidum, Pleutrotus sajor-caju, Agaricus bitorquis* (A_{61}), *Agaricus bitorquis* (J_{77}), *Agaricus bitorquis* (A_{67}) and *Agaricus bitorquis* (A_{65}) were cultivated in liquid broth (Vogel medium)[11,14]. The composition of growth medium (g/L) was as follows: KH_2PO_4 (5.0), D-glucose (5.0), $MgSO_4 \cdot 7H_2O$ (0.2), NH_4NO_3 (2.0), peptone (2.0), trisodium citrate (2.5), yeast extract (1.0) and $(NH_4)_2SO_4$ (4.0). For screening studies growth medium pH was maintained at 4.5 using 0.1N NaOH and 0.1N HCl before autoclaving at 121 ºC for 15 min. Inculcated flasks were incubated at 30 °C for 7 days at 150 rpm on a shaking incubator[15,16].

Microorganism, media and immobilization: From eleven white rot fungi used in initial study, three white-rot fungi *viz.*, *Pleutrotus sajor-caju, Agaricus bitorquis* and *Ganoderma lucidum* were selected for biosorption experiments on the basis of previous studies[16]. For biosorption studies fungal mycelia were killed by autoclaving at 121ºC for 0.5 h. For immobilization of dead fungal mycelia, 1 g of each biomass was mixed in a high speed blender (Panasonic model MJ-W176P) with 2 g of Na-alginate dissolved in 100 mL of double distilled water. The beads of 3.55 mm size were formed by introducing mixture into 0.1M $CaCl_2$ solution using a narrow sized burette. For bioremediation studies, immobilization of basidiospores of each live fungus *via* entrapment was carried out using following procedure: Two grams of Na-alginate were dissolved in 90 mL of double distilled water and then mixed with 10 mL fungal spore suspension having 1×10^9 basidiospore/mL. The whole mixture was drop wise introduced into 0.1M $CaCl_2$ using a narrow sized burette. The size of beads was found to 3.55 mm. Calcium chloride solution was constantly stirred to prevent aggregation of Ca-alginate beads. Thus formed fungal beads were washed thrice using 300 mL of sterilized double distilled water. Total dry weights of fungal growth on/in beads were determined using an analytical balance after drying overnight in an electrical oven maintained at 60 ºC.

Biosorption studies: During biosorption studies of white rot fungi fixed volume of metal solution (100 mL) was used to evaluate the effect of various experimental parameters. The effect of pH was checked from pH 1 to 10. The dosage of white rot fungi was varied from 0.05 to 0.3 g/100 mL. To check the influence of initial metal concentration on biosorption process, the metal concentration was varied from 25 to 1000 mg/L. The contact time of white rot fungi with aqueous solution was varied from 15 min to 240 min. The dependency of biosorption on solution temperature was evaluated from 30 to 60 ºC range. The influences of shaking speed (0 to 200 rpm), multimetal concentration (0 to 400 mg/L) and co-metal ions (0 to 400 mg/L) were also optimized during the present study. The flasks were agitated on an orbital rotating shaker (PA 250/25. H) at constant shaking speed in each batch experiment. On completion of each experiment, the sample flasks were removed from the orbital shaker and the aqueous solutions were filtered using Whatman filter paper No. 40 to separate them from biomass[1]. The column studies were carried out by varying contact time interval from 15 to 240 min.

Desorption: Desorption of metal ions from biomass of white rot fungi was carried out using 0.1N EDTA, CH_3COOH, H_2SO_4, HCl and NaOH.

$$\text{Desorption ratio (\%)} = \frac{\text{Amount of desorbed metal ions desorbed}}{\text{Amount of adsorbed metal ions}} \times 100$$

Metal solutions: Stock solutions (1000 mg/L) of Cr(III) and Cr(VI) were prepared using 7.695 g of $Cr_3N_3O_9 \cdot 9H_2O$ and 5.6575 g of $K_2Cr_2O_7$ in 1 L of double distilled water. The procedure of stock solution preparation was as follows: the measured amount of metal was initially dissolved in 100 mL of deionized distilled water and subsequently solution was diluted up to 1000 mL using double distilled water. Metal solutions of required concentrations were prepared by appropriate dilution of the stock solution (1000 mg/L) with double distilled water.

Determination of the metal contents in the solutions: The concentration of metal in the aqueous/industrial solutions before and after the equilibrium reached was determined by using a Perkin-Elmer AAnalyst 300 atomic absorption spectrometer 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 HNO_3 (10 % v/v) over-

night before experiment. Before use, polypropylene flasks and glassware rinsed several times with double distilled water.

Metal uptake: The concentration difference method was used to calculate metal uptake by white rot fungi. 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 white rot fungi q (mg/g) was calculated from the mass balance as follows:

$$q = \frac{(C_o - C_e)V}{1000W}$$

where, V = volume of the solution (mL) and W = biosorbent mass (g).

Biomass analysis: FTIR spectra were taken by Bio-Rad FTS-135 spectrometer using a KBr window within the range 4000-400 cm^{-1}. SEM and EDAX microscopic analysis were collected a scanning electron microscope model JEOL, JSM-5910, Japan.

Statistical analysis: Standard deviation was calculated for three independent determinations for each variable[17]. The linear regression analysis were carried out to calculate the parameters of Langmuir adsorption isotherm, Freundlich adsorption isotherm, pseudo first order kinetic model and pseudo second order kinetic model.

RESULTS AND DISCUSSION

Influence of initial pH: Solubility of metal ions as well as ionization of functional groups is dependent on the solution pH. The main components of fungal cells are polysaccharides including proteins, melanin and lipids. Fungal cells were characterized in past and it was found they have several functional groups *viz.*, carboxyl, amino, sulfydryl, phoshphate and thiol groups[1,13]. White rot fungi used in the present study found to have ionizable groups on cell surface. The effect of pH on uptake of Cr(III), Cr(VI) uptake by native and immoblized *Pleurotus sajor-caju, Agaricus bitorquis* and *Ganoderma lucidum* is presented in Figs. 1 and 2. The uptake of metal ions increased after immobilization of dead white rot fungi. This might be due to more proper interaction of entrapped dead fungal biomass in alginate framework with metal solution than native biomass. Iqbal and Saeed[18] found that under identical conditions immobilized fungal biomass removed 41.93 % more metal than free fungal biomass. At lower pH, functional groups present on biomass cell wall were protonated and have positive charge. Main essential requirement of an industrial biosorption system is that biosorbents could be utilized both in batch and continuous setups. During the continuous industrial process there is a need of proper utilization technique since biomass in free form are not be used. Free biomass cells have small particle size and low mechanical strength and excessive hydrostatic pressures are generated during maintenance of suitable flow rate. Disintegration of free biomass cells occurred at high pressures. The solution to these problems is immobilization of biomass. It provides high biomass loading, minimal clogging and better reusability in continuous flow systems. The Cr(VI) uptake was maximum[5] at pH 2 due to presence of its negatively charged species (CrO_4^{2-} and $Cr_2O_7^{2-}$) at this pH. Kratochvil *et al.*[19] found that 2.0-2.5

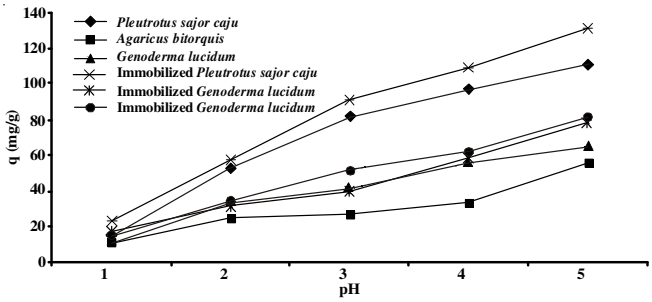

Fig. 1. Cr(III) uptake by native and immobilized white rot fungi

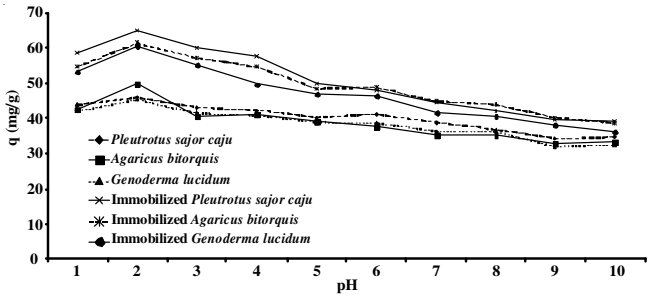

Fig. 2. Cr(VI) uptake by native and immobilized white rot fungi

pH was optimum for Cr(VI) removal using seaweed *Sargassum biomass*. Chen and Yang[20] observed that Cr(VI) removal was optimal at pH 2. The Cr(III) ions were present as a positive ion at low pH values. Hence their adsorption decreases with decrease in pH due to increase in positive charge density on biomass cell surface. On increasing solution pH, overall negative charge on biomass cell surface was developed. Subsequently, Cr(VI) biosorption showed a decreasing trend while removal of Cr(III) by dead white rot fungi biomass increased[14]. The optimum pH for Cr(III) and Cr(VI) by native/ immobilized *Pleurotus sajor-caju, Agaricus bitorquis* and *Ganoderma lucidum* was found to be 5 and 2, respectively. After pH 5 for Cr(III), insoluble metal hydroxide precipitated from aqueous solutions. The dependency of metal uptake phenomenon on solution pH was also noted by Tan and Cheng[21] for Cr^{3+}, Ni^{2+} and Zn^{2+} uptake using *Penicillium chrysogenum* mycelium.

Effect of biosorbent concentration: Both biomass and initial metal concentrations are considered important parameters for effective biosorption studies as these determine sorbent and sorbate ratio in solution[22,23]. The effect of biosorbents dose on metal biosorption by dead immobilized white rot fungi was studied by varying dosage from 0.05 to 0.3 g/100 mL (Fig. 3). Increase in biomass dose in a fixed volume of solution (100 mL) at constant pH (5), initial metal concentration (100 mg/L) and temperature (30 ºC) reduced the metal sorption capacity of biomass. This decrease in uptake capacity of biosorbents can be attributed to poor utilization of biomass/ lower efficiency at higher biomass concentrations. At higher biomass dosage agglomeration of biomass cells occurs which reduce inter-cellular distance significantly. There is a need of optimal inter-cellular distance to ensure optimal electrostatic interaction between fungal cells. Optimal inter-nuclear distance is a significant factor in metal biosorption studies. Similar type of results are reported in literature by several workers[22,24,25].

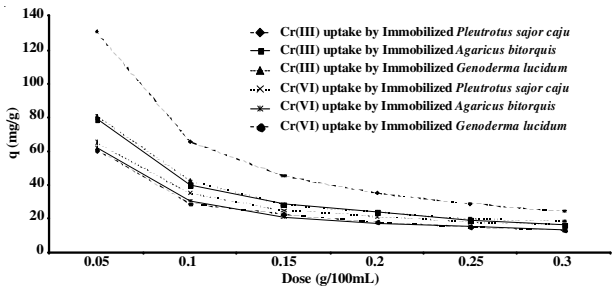

Fig. 3. Effect of biosorbents concentration on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

Effect of initial metal concentration: Effect of initial metal concentration on biosorption of Cr(III) and Cr(VI) by immobilized *Pleurotus sajor-caju, Agaricus bitorquis* and *Ganoderma lucidum* dead biomass was studied inconcentration range 25 to 1000 mg/L (Fig. 4). Biosorption of both metal ions increased as the concentration was increased from 25 to 200 mg/L. A further increase in metal ion concentration in solution not showed any significant increase in metal biosorption. Ceribasi and Yetis[26] used resting cells of *Phanerochaete chrysosporium* for biosorption of Ni(II) and Pb(II) and found that the metal uptake capacity of fungus increased as the initial concentration of Ni(II) and Pb(II) increased in the solution. This might be due to increase in number of competing ions for a fixed number of adsorption sites[27]. This metal sorption characteristic clearly demonstrated that initial metal concentrations controls the biomass cell surface saturation. At low concentrations, metal uptake by biomass was easy as adsorption sites can be taken up quickly. However, at higher concentrations intraparticle diffusion is needed and more hydrolyzed ions will diffuse into biomass cell at a comparatively slow rate[28]. The Cr(III) and Cr(VI) uptake capacity (mg/g) of immobilized dead white rot fungi was in following order: *Agaricus bitorquis* > *Pleutrotus sajor-caju* > *Ganoderma lucidum*. All white rot fungi adsorbed Cr(III) more in comparison to Cr(VI).

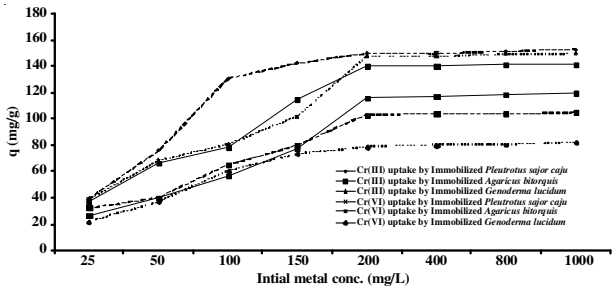

Fig. 4. Effect of initial metal concentration on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

The biosorption data of Cr(III) and Cr(VI) were fitted to most commonly applied Langmuir and Freundlich adsorption isotherms. Langmuir adsorption isotherm model fitted well to the adsorption data of Cr(III) and Cr(VI) as represented by its high value of correlation coefficient (R^2) (Table-1). Secondly, the estimated q_e value from Langmuir equation agreed well to experimental q_e value. These both facts confirmed that data fitted well to Langmuir adsorption isotherm model. Ozer and Ozer[29] also noted the fitting of Langmuir adsorption isotherm model to Pb(II), Ni(II) and Cr(VI) uptake by inactive *Saccharomyces cerevisiae*.

Biosorption kinetics: A kinetic study with predefined time intervals was designed to study the effect of contact time on biosorption of Cr(III) and Cr(VI) by immobilized *Pleurotus sajor-caju*, *Agaricus bitorquis* and *Ganoderma lucidum* dead biomass at various solution temperatures (Figs. 5a-e). The effect of contact time on metal uptake showed almost similar type of trend at all temperatures. The metal uptake by immobilized dead biomass occurred in two phase. First rapid phase completed within 0.5 h, followed by a slow metal adsorption phase till the equilibrium. Equilibrium in metal uptake was reached within 2 h of contact. Equilibrium time of 2 h for Cr(VI) uptake using free and CMC immobilized *Lentinus sajor-caju* was also observed by Arica and Bayremoglu[30]. The rapid sorption attributed to initial sorption phase was probably due to extra cellular binding of metal ions. The slow sorption phase was due to intracellular metal ion binding. The biosorption of Cr(III) and Cr(VI) by immobilized *Pleurotus sajor-caju*, *Agaricus bitorquis* and *Ganoderma lucidum* dead biomass was very rapid as compared some previous studies[4,9]. It might be due to presence of greater number of sorption sites on surface of immobilized dead white rot fungi biomass. These sorption increased sorption rate as well as improved metal

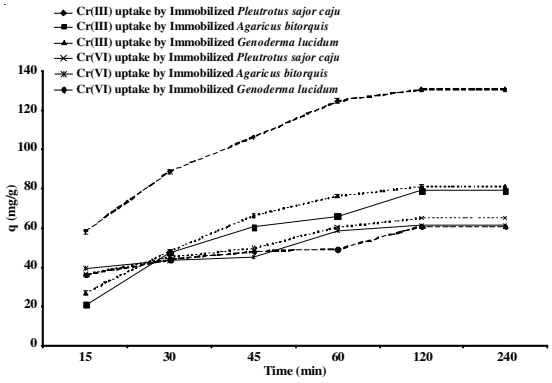

Fig. 5a. Effect of time on Cr(III) and Cr(VI) uptake by immobilized white rot fungi at 30 ºC

TABLE-1
A COMPARISON BETWEEN LANGMUIR AND FREUNDLICH ADSORPTION ISOTHERM PARAMETERS FOR METAL UPTAKE BY IMMOBILIZED DEAD WHITE ROT FUNGI BIOMASS

Fungus	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
Agaricus bitorquis	Cr(III)	153.84	1.15×10^{-1}	0.9999	152.74	189.52	42.92	0.2175	0.7225
	Cr(VI)	131.58	5.44×10^{-2}	0.9988	127.92	159.70	27.62	0.2558	0.7823
Pleutrotus sajor-caju	Cr(III)	144.92	4.55×10^{-2}	0.9985	141.88	170.78	30.72	0.2510	0.8609
	Cr(VI)	125.00	6.82×10^{-2}	0.9997	122.36	148.94	33.39	0.2158	0.8386
Ganoderma lucidum	Cr(III)	153.85	3.69×10^{-2}	0.9976	149.58	176.70	30.90	0.2553	0.8813
	Cr(VI)	131.58	3.51×10^{-2}	0.9984	127.28	162.32	19.72	0.3081	0.7849

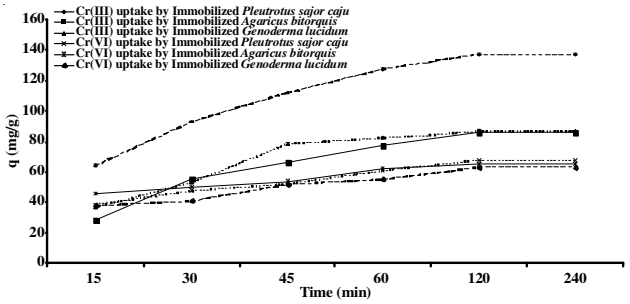

Fig. 5b. Effect of time on Cr(III) and Cr(VI) uptake by immobilized white rot fungi at 40 ºC

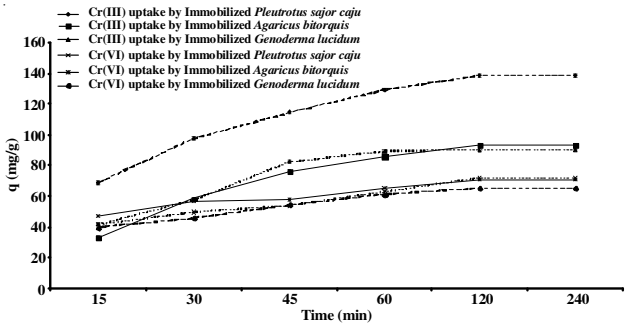

Fig. 5c. Effect of time on Cr(III) and Cr(VI) uptake by immobilized white rot fungi at 50 ºC

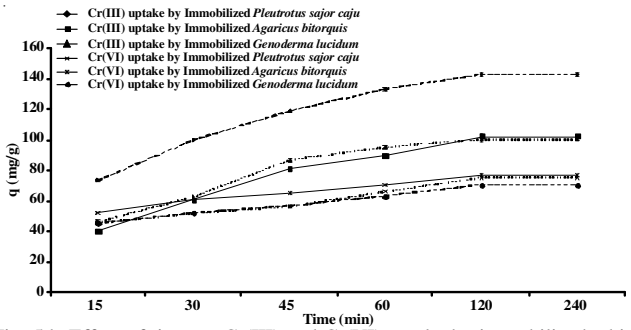

Fig. 5d. Effect of time on Cr(III) and Cr(VI) uptake by immobilized white rot fungi at 55 ºC

binding capacity significantly. This rapid biosorption property of white rot fungi is one of the most important factors required for an economical effluent treatment process.

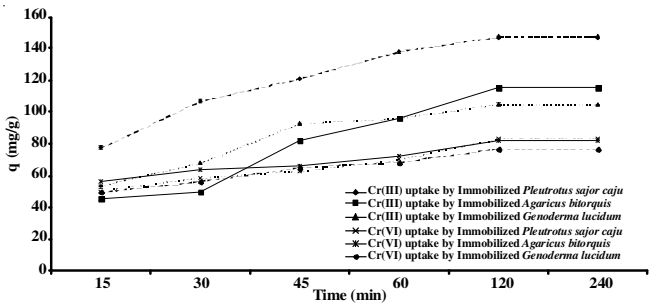

Fig. 5e. Effect of time on Cr(III) and Cr(VI) uptake by immobilized white rot fungi at 60 ºC

To describe the behaviour of batch as well as continuous biosorption processes, the mathematical models are very useful for scale up considerations and for process optimization. Pseudo first and second order models were applicable to the results of the present study. The contact time data obtained at 30-60 ºC was subjected to kinetic modeling. The graphs of kinetic models at 30 ºC are only presented here. Whereas kinetic parameters of pseudo first and second order kinetic models at all temperatures were calculated and given below in Tables 2 and 3. From the value of correlation coefficient (R^2) and estimated qe value it is suggested that best fit model was pseudo second order kinetic model.

Effect of temperature: The influence of solution temperature on the Cr(III) and Cr(VI) uptake by immobilized *Pleurotus sajor-caju, Agaricus bitorquis* and *Ganoderma lucidum* dead biomass was investigated at five different temperatures (Fig. 6). The results indicated that the biosorption of Cr(III) and Cr(VI) depend on temperature. The Cr(III) and Cr(VI) uptake by immobilized *Pleurotus sajor-caju, Agaricus bitorquis* and *Ganoderma lucidum* dead biomass increased as temperature was increased suggesting that process was endothermic in nature.

Thermodynamic parameters: The Gibbs free energy (ΔG^o), enthalpy (ΔH^o) and entropy (ΔS^o) can play very vital role in designing reactors for commercial biosorption process[10]. The ΔG^o for metal biosorption process was calculated from following equation:

TABLE-2
A COMPARISON BETWEEN PSEUDO FIRST AND SECOND ORDER KINETIC MODELS FOR Cr(III) UPTAKE BY IMMOBILIZED DEAD WHITE ROT FUNGI VARIOUS TEMPERATURES

Fungus	Temp. (ºC)	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
Agaricus bitorquis	30	91.45	3.38×10^{-2}	0.9882	78.88	140.84	4.75×10^{-4}	0.9959
	40	105.90	3.90×10^{-2}	0.9479	65.30	147.05	4.60×10^{-4}	0.9989
	50	131.06	4.60×10^{-2}	0.9924	93.30	103.09	4.80×10^{-4}	0.9913
	55	113.60	3.60×10^{-2}	0.9911	102.42	113.63	4.10×10^{-4}	0.9946
	60	130.79	3.00×10^{-2}	0.9228	115.34	133.33	2.38×10^{-4}	0.9817
Pleutrotus sajor-caju	30	192.17	5.30×10^{-2}	0.9350	130.62	91.74	3.52×10^{-4}	0.9795
	40	156.80	4.30×10^{-2}	0.9748	137.54	96.15	4.60×10^{-4}	0.9908
	50	145.04	4.30×10^{-2}	0.9765	138.44	147.05	5.20×10^{-4}	0.9979
	55	148.79	4.30×10^{-2}	0.9750	142.88	151.51	5.20×10^{-4}	0.9980
	60	144.47	4.20×10^{-2}	0.9619	147.26	156.25	3.36×10^{-6}	0.9983
Ganoderma lucidum	30	144.24	5.40×10^{-2}	0.9686	80.80	90.09	5.07×10^{-4}	0.9874
	40	123.68	5.00×10^{-2}	0.9549	86.18	94.33	6.70×10^{-4}	0.9926
	50	290.87	8.90×10^{-2}	0.9202	90.22	96.15	8.05×10^{-4}	0.9937
	55	149.38	5.40×10^{-2}	0.9633	100.22	107.52	6.29×10^{-4}	0.9949
	60	106.43	4.30×10^{-2}	0.9458	104.42	111.11	6.84×10^{-4}	0.9969

TABLE-3
A COMPARISON BETWEEN PSEUDO FIRST AND SECOND ORDER KINETIC MODELS FOR Cr(VI) UPTAKE BY IMMOBILIZED DEAD WHITE ROT FUNGI VARIOUS TEMPERATURES

Fungus	Temp. (°C)	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
Agaricus bitorquis	30	59.53	3.70×10^{-2}	0.8798	61.26	64.53	1.27×10^{-3}	0.9962
	40	42.34	2.70×10^{-2}	0.9479	65.30	68.02	1.78×10^{-3}	0.9989
	50	39.72	3.20×10^{-2}	0.9015	70.78	61.34	2.27×10^{-4}	0.9992
	55	37.75	2.85×10^{-2}	0.9813	76.54	79.36	1.51×10^{-3}	0.9995
	60	35.10	2.00×10^{-2}	0.9485	81.92	85.47	1.17×10^{-4}	0.9989
Pleutrotus sajor-caju	30	29.95	1.70×10^{-2}	0.9487	65.02	69.44	1.06×10^{-3}	0.9976
	40	50.00	3.00×10^{-2}	0.9345	67.90	72.46	1.02×10^{-3}	0.9983
	50	47.86	2.50×10^{-2}	0.9333	72.12	76.92	9.0×10^{-4}	0.9980
	55	47.11	2.48×10^{-2}	0.9048	75.20	80.0	8.20×10^{-4}	0.9977
	60	44.97	1.90×10^{-2}	0.9731	82.84	88.49	7.90×10^{-4}	0.9991
Ganoderma lucidum	30	56.27	4.20×10^{-2}	0.7188	60.72	64.51	1.10×10^{-3}	0.9980
	40	44.23	2.70×10^{-2}	0.8398	63.16	67.11	1.10×10^{-3}	0.9980
	50	55.64	4.0×10^{-2}	0.9344	65.16	68.96	1.33×10^{-4}	0.9985
	55	39.98	2.65×10^{-2}	0.9650	70.68	74.62	1.21×10^{-3}	0.9989
	60	42.61	2.60×10^{-2}	0.9856	76.76	80.64	1.18×10^{-4}	0.9991

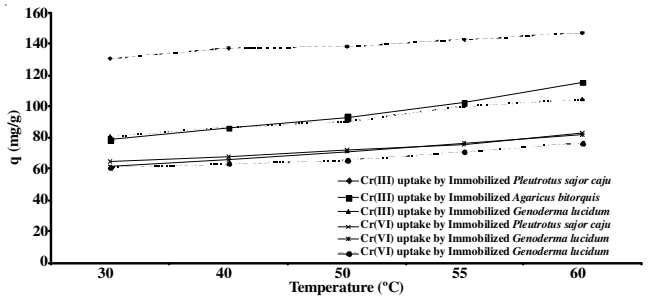

Fig. 6. Effect of temperature on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

$$\Delta G^\circ = -RT \ln K_c$$

where, K_c is equilibrium constant, R gas constant (8.314 kJ mol^{-1} K^{-1}) and T absolute temperature (K).The ΔG° value for Cr(III) and Cr(VI) uptake by three white fungi was spontaneous from 25-800 mg/L concentration range as indicated by negative vales of ΔG° with few exceptions (Table-4). The results clearly demonstrates that as the initial concentration of metal ion rises in the solution, the metal uptake process by immobilized dead white rot fungi become less spontaneous.

The values of ΔH° and ΔS° were calculated by the use of following equation:

$$\ln K_c = (\Delta S^\circ/R) - (\Delta H^\circ/RT)$$

and

$$K_c = C_a/C_e$$

where, C_a adsorbate adsorbed (mg/L), C_e equilibrium concentration (mg/L). The values of both ΔH° and ΔS° for Cr(III) and Cr(VI) uptake by white rot fungi were positive (Table-5). The positive ΔH° values indicate that the metal uptake process by immobilized dead white rot fungi was endothermic in nature. The positive ΔS° values demonstrated that there is an increase in degree of randomness after metal biosorption. These observations confirm that the metal uptake process by immobilized dead white rot fungi was energetically feasible.

Effect of shaking speed: The effect of shaking speed on Cr(III) and Cr(VI) uptake by immobilized *Pleurotus sajor-caju, Agaricus bitorquis* and *Ganoderma lucidum* dead biomass uptake was evaluated by varying the shaking speed from 0 rpm (no shaking) to 200 rpm (Fig. 7). Increase in agitation speed increased Cr(III) and Cr(VI) uptake by immobilized

TABLE-4
CALCULATED FREE ENERGY (ΔG°) PROFILE FOR Cr(III) AND Cr(VI)

Fungus	Metal conc.	ΔG° for Cr(III)	ΔG° for Cr(VI)
Immobilized *Pleutrotus sajor-caju*	25	-9.502090	-8.477850
	50	-8.686520	-5.847550
	100	-7.395840	-5.074350
	150	-5.481030	-5.012020
	200	-4.512890	-3.878850
	400	-2.095560	-1.608390
	800	-0.104210	0.366907
	1000	0.478435	0.957443
Immobilized *Agaricus bitorquis*	25	-9.015260	-8.865710
	50	-7.516470	-7.076450
	100	-4.721030	-5.507060
	150	-4.642800	-4.122320
	200	-4.241090	-3.709310
	400	-1.907200	-1.464010
	800	0.079104	0.492816
	1000	0.679006	1.0768640
Immobilized *Ganoderma lucidum*	25	-9.251680	-6.920570
	50	-7.767140	-5.328040
	100	-4.821880	-4.785880
	150	-4.185880	-4.631300
	200	-4.454070	-3.808480
	400	-2.049300	-1.543860
	800	-0.074210	0.410177
	1000	0.535407	0.970940

TABLE-5
CALCULATED ENTHALPY (ΔH°) AND ENTROPY (ΔS°) VALUES FOR METAL ADSORPTION BY WHITE ROT FUNGI

Immobilized fungus	Metal	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
Pleutrotus sajor-caju	Cr(III)	9.85	56.90
Agaricus bitorquis		18.99	77.77
Ganoderma lucidum		13.05	58.71
Pleutrotus sajor-caju	Cr(VI)	12.23	52.25
Agaricus bitorquis		9.06	41.66
Ganoderma lucidum		9.66	44.48

Pleurotus sajor-caju, Agaricus bitorquis and *Ganoderma lucidum* dead biomass. The results showed that the increase in shaking speed, improves the diffusion of Cr(III) and Cr(VI) toward the fungal surface[31]. Similar results were reported by Zubair *et al.*[5] during Cr(III) and Cr(VI) uptake using *Citrus reticulate* waste biomass. The results indicate that biosorption capacity increases with increase in shaking speed.

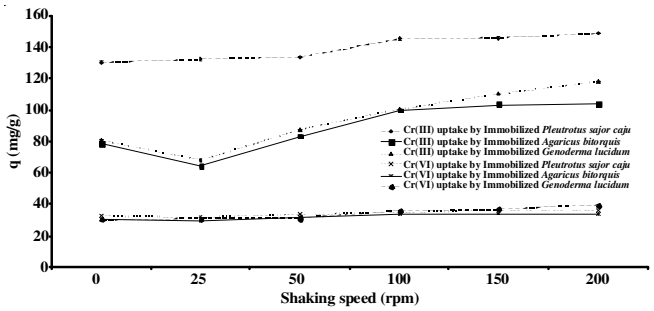

Fig. 7. Effect of shaking speed on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

Effect of other ions presence: For industrial application of biosorption phenomenon the effect of concentration of other ions on the uptake of heavy metal ions must be evaluated. The experiments were conducted to determine the effect of common cations such Na^+, Ca^{2+}, Al^{3+} (Figs. 8a-c) and anions such as Cl^-, NO_3^{2-}, SO_4^{2-} and CH_3COO^- on Cr(III) and Cr(VI) uptake by white rot fungi dead biomass. The concentrations of competing ions were varied from 0 to 400 mg/L. Within the examined concentration range, the uptake of Cr(III) and Cr(VI) was effected by the presence of co-metal cations and was unaffected by the presence of anions. Because anions did not exerted any significant effect on the biosorption of Cr(III) and Cr(VI) by white rot fungi (data not shown). The inhibition effect exerted by metal cations on Cr(III) and Cr(VI) biosorption by dead white rot fungi suggest that they have competed for similar type of adsorption sites. The inferences caused by cations in metal biosorption were in the following order: $Al^{3+} > Ca^{2+} > Na^+$. It can be noted from results obtained that greater the charge on competing cation greater the interference caused by it. Similar types of results are reported in literature by several authors[32-35].

The effect of presence of Cu(II) and Pb(II) on Cr(III)/Cr(VI) uptake using three immobilized dead white rot fungi was evaluated by varying the concentration of Cu(II) and Pb(II) from 0 to 400 mg/L at a fixed concentration of Cr(III) and Cr(VI) (Figs. 8d-e). It was not possible to check the effect of presence of Cr(III) on Cr(VI) uptake or *vice-versa* due to formation of insoluble precipitate during the study. The uptake of metal under study decreased almost linearly with increase in the concentration of other metal ion. The significant decrease in metal uptake on addition of competitive metal ion clearly demonstrated that both metal ions competed for similar type of adsorption sites present on biomass surface[36,37]. Kovacevic *et al.*[38] also observed that metal uptake capacity of fungus is affected in multi component system. These authors found that *Aspergillus niger* adsorption affinity for Cu^{2+}, Zn^{2+} and Ni^{2+} ions in single and only for Cu^{2+} and Zn^{2+} in multi-components solution. Han *et al.*[39] also found that the amount of one metal

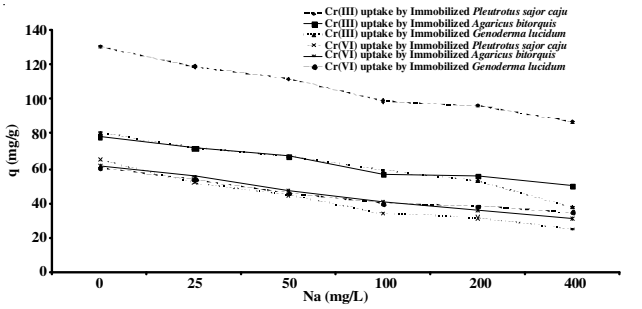

Fig. 8a. Effect of Na concentration in solution on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

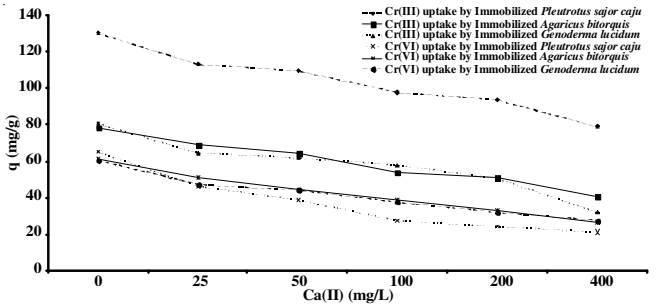

Fig. 8b. Effect of Ca(II) concentration in solution on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

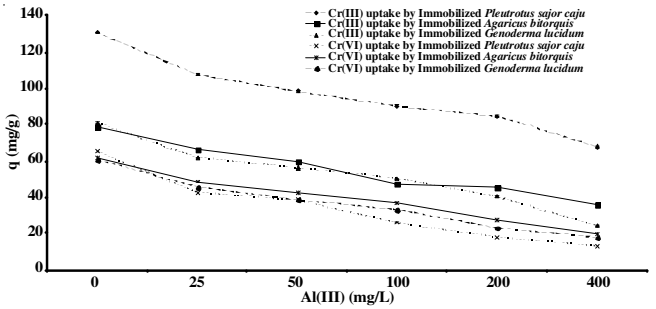

Fig. 8c. Effect of Al(III) concentration in solution on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

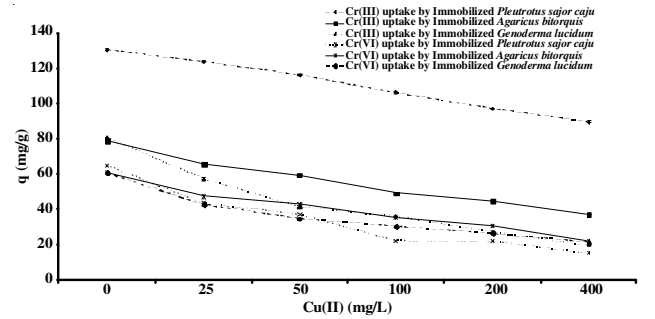

Fig. 8d. Effect of Cu(II) concentration in solution on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

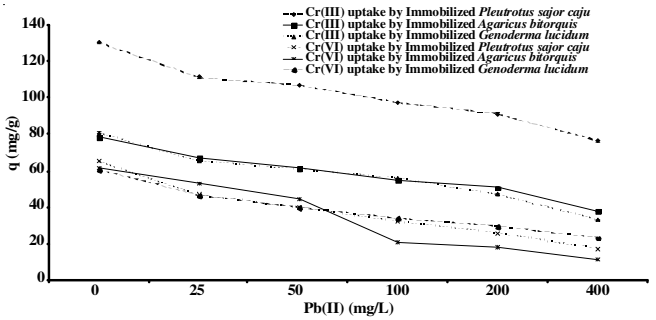

Fig. 8e. Effect of Pb(II) concentration in solution on Cr(III) and Cr(VI) uptake by immobilized white rot fungi

TABLE-6
A COMPARISON BETWEEN PSEUDO FIRST AND SECOND ORDER KINETIC MODELS FOR Cr(III) AND Cr(VI) UPTAKE BY IMMOBILIZED DEAD WHITE ROT FUNGI BIOMASS IN COLUMN SETUP

Fungus	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
Pleutrotus sajor-caju	Cr(III)	81.58	4.88×10^{-2}	0.8993	123.42	128.20	1.20×10^{-3}	0.9986
	Cr(VI)	54.51	3.47×10^{-2}	0.9076	59.56	64.10	9.90×10^{-4}	0.9973
Agaricus bitorquis	Cr(III)	73.41	4.05×10^{-2}	0.9455	73.00	78.74	8.51×10^{-4}	0.9965
	Cr(VI)	23.55	3.08×10^{-2}	0.8955	52.76	54.35	2.72×10^{-3}	0.9994
Ganoderma lucidum	Cr(III)	47.62	4.60×10^{-2}	0.9015	75.70	78.12	2.20×10^{-3}	0.9995
	Cr(VI)	36.08	4.90×10^{-2}	0.9698	51.02	52.63	3.19×10^{-3}	0.9995

ion adsorbed decreased significantly with increase in the amount of competing metal ion.

Column studies: A pyrex glass column having 75 cm height and 1.8 cm internal diameter was packed with 10 g of immobilized dead white rot fungi biomass. The obtained bed depth and bed volume were 50 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 of the glass column was filled with glass beads. At the bottom of column glass wool was placed between the glass beads and the biomass beads. The bottom of column was sealed using a rubber stopper with a single bore. The connection in 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 optimum pH was maintained during column study. The concentration of metal ions in the eluent phase was determined after predetermined time intervals (Fig. 9). The uptake of metals under study was more in batch setup in comparison to continuous mode. Immobilized dead *Pleutrotus sajor-caju* found to have more Cr(III) and Cr(VI) uptake capacity than other two white rot fungi. The contact time data was fitted using pseudo 1st and 2nd order kinetic models. The pseudo second kinetic models fitted well to experimental data of column study as it had higher value of correlation coefficient (R^2). Secondly, value of estimated q_e from pseudo second order kinetic model agreed well to experimental q_e value (Table-6).

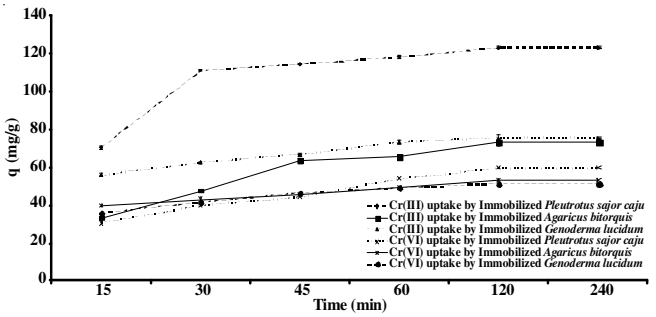

Fig. 9. Column studies for Cr(III) and Cr(VI) uptake by immobilized white rot fungi

Desorption: For the proper utilization of biomass for metal biosorption, the regeneration studies must be carried out. The best eluent could be that one which recover the retained metal and do not affect reusability of the biomass. Several desorbing agents are reported in literature[22,40,41]. The tested eluents were inorganic and organic acid and complexing agents[42]. In the present study, five eluents were tested: EDTA, acetic acid, sulphuric acid, hydrochloric acid and sodium hydroxide (Fig. 10). Amount of metal desorbed from biomass surface was calculated from following equations:

$$q_{des} = C_{des} \cdot V/m$$

where, q_{des} = desorbed amount of metal (mg/g); V = volume of solution taken (L); m = weight of biosorbent (g); C_{des} = concentration of metal in eluent.

$$\text{Desorption (\%)} = [q_{des}/q]/100$$

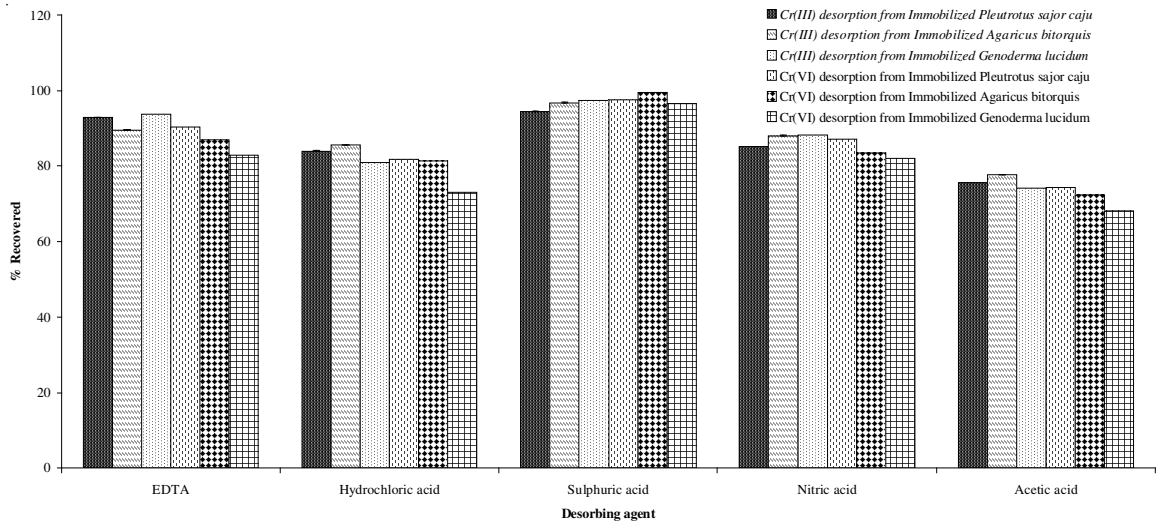

Fig. 10. Desorption studies for Cr(III) and Cr(VI) uptake by immobilized white rot fungi

Acid elute the metal from biomass surface by lowering the pH and creating a net positive charge on biomass surface. Sodium hydroxide removed metal from biomass surface by forming its hydroxide. EDTA is a strong chelating agent for heavy metals. EDTA replaces the amine groups present on the cell surface and forms complex with metal ions[43]. After desorption (using all eluents) the biomass was successfully used in fifteen sorption-desorption cycles without any considerable loss in its metal uptake capacity. Sulphuric acid was found as the best eluent for metal recovery from dead fungal biomass. Bai and Abraham[44] found that *Rizopus nigricans* biomass immobilized using polysulfone can be regenerated and reused for more than 25 cycles with 75-78 % efficiency.

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