A comparative study on the removal and recovery of hexavalent chromium from tannery wastewater using an isolated strain *Aspergillus proliferans LA* and a known strain *Aspergillus terreus*

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Abstract
Isolation of a potent microorganism from the soil near tanneries and a comparative analysis of the removal and recovery of different concentrations of hexavalent chromium using known strain Aspergillus terreus and isolated strain Aspergillis proliferansLA are done. The isolated fungal species from the chromium contaminated soil sites located in the industrial area of Kanpur, U.P. were tested for its potential for the removal of hexavalent chromium from wastewater streams. The experiments were conducted comparing the biosorption efficiency of the isolated species Aspergillus proliferansLA and Aspergillus terreus by varying the initial Cr (VI) concentration and media constituents.

The highest removal efficiency of 99.19% was shown by the isolated species Aspergillus proliferansLA for an initial Cr (VI) concentration of 10ppm followed by increasing the concentration to 50ppm whereas Aspergillus terreus showed 78.43% removal of hexavalent chromium. This was then followed by desorption using 0.8M NaOH and 75.12% hexavalent chromium was desorbed from the cells. The isolated strain Aspergillus proliferansLA was seen to show better removal and recovery results than the known strain Aspergillus terreus.

Keywords: Biosorption, Chromium, *Aspergillus proliferansLA*, *Aspergillus terreus*, PDA Potato Dextrose Agar, PM Particulate matter.

Introduction

The heavy metals are found naturally in the earth's crust and have density higher than that of water. The major problem of our country these days is the heavy metal pollution in air, water and soil and has the potential of bioaccumulation and biomagnifications which result in cumulative hazardous effects to humans, animals, plant and aquatic life. These heavy metals when present in the environment cause a number of adverse effects and affect all the spheres of the atmosphere i.e. hydrosphere, lithosphere, biosphere and atmosphere.[44] The presence of heavy metals causes adverse impacts in the human body, Chromium affects the skin, lungs, kidneys, liver, brain, pancreas, testes, gastrointestinal and reproductive, Arsenic affects the skin, lungs, brain, kidneys, liver, metabolic, cardiovascular, immunological and endocrine.

Cadmium affects the bones, liver, kidney, lungs, testes, brain, immunological and cardiovascular. Lead affects the bones, liver, kidneys, lungs, brain, spleen, immunological, haematological, cardiovascular and reproductive. Mercury affects the brain, lungs, kidney, liver, immunological, cardiovascular, endocrine and reproductive and copper affects the liver, brain, kidney, cornea, gastrointestinal, lungs, immunological and haematological.[8]

The water contamination actually affects all the organisms of the ecosystem. The accumulation of heavy metals in the soft tissues of the body when these are not metabolized, causes metal toxicity.[28]

The main source of chromium into the ecosystem is through the industrial activities like leather tanning, wood preservation, fungicide production, textile dying, metal electroplating and fertilizer production. The permissible limits for the drinking water quality prescribed by World Health Organization (WHO), United States Environmental Protection Agency (USEPA), Indian Standard Institution (ISI), Indian Council of Medical Research (ICMR) and Central Pollution Control Board (CPCB) is given in the table 1.[24]

There are various conventional methods to remove heavy metals from waste water streams like chemical precipitation, reduction, chemical oxidation, lime coagulation, ion exchange, solvent extraction, reverse osmosis and electro-dialysis.[36]

Several species of fungi such as *Aspergillus awamori, C. tamariscifolia, Aspergillus flavus, Aspergillus gracilis, Aspergillus penicillioides, Aspergillus penicillioides, Aspergillus restrictus, Sterigmatomyces halophilus* etc. have been analyzed for their potential in removal of various concentrations of hexavalent form of chromium from wastewater. The aim of this study was to isolate a fungal species which aids in the removal of hexavalent chromium. In isolation of the fungal species, initially 20ppm Cr(VI) concentration was maintained for acclimatization of the species. The removal efficiency of the isolated species *Aspergillus proliferansLA* and *Aspergillus terreus* was compared in five different experimental units.

Table 1
The permissible limits of heavy metal concentration for the drinking water quality

Heavy metal	Permissible limit				
(mg/l)	WHO	USEPA	ISI	CPCB	ICMR
Iron	0.1	-	0.3	1.0	1.0
Copper	1.0	1.3	0.05	1.5	1.5
Mercury	0.001	0.002	0.001	No relaxation	0.001
Cadmium	0.005	0.005	0.01	No relaxation	0.01
Arsenic	0.05	0.05	0.05	No relaxation	0.05
Lead	0.05	-	0.10	No relaxation	0.05
Zinc	5.0	-	5.0	15.0	0.10
Chromium	0.1	-	0.05	No relaxation	-

The kinetics and thermodynamics analysis were carried followed by desorption of hexavalent chromium using 0.8M NaOH which promotes reusing of the biomass as well as the desorbed chromium for use in the tanning process.

Material and Methods

Isolation of microorganism: The soil samples were collected from the highly chromium contaminated areas around the dumping sites of tanneries. The isolation of the fungal strain was performed using serial dilution method. The medium used for isolation was potato dextrose agar Petri plates with initial chromium concentration of 20ppm. These PDA plates were then incubated at 30˚C for four days. After further purification, five colonies were obtained of which three appeared to be fungal on the basis of their morphological characteristics.

The purified fungal colonies obtained were transferred to agar slants for further analysis. The MALDI-TOFF testing was then performed. On the basis of the further analysis on different concentrations of chromium, one fungal isolate was selected for further analysis. The colonies in the form of active slants were then sent to NCIM-NCL, Pune for 18s rRNA sequencing. The extraction of chromosomal DNA of the isolated species was done using spin column kit (HiMedia, India). The amplification of the 18SrRNA gene (1500bp) was carried out using polymerase chain reaction in a thermal cycler followed by purification using Exonuclease I – Shrimp Alkaline Phosphatase (Exo-SAP). The amplicons were then sequenced using the Sanger method in ABI 3500xl genetic analyzer (Life Technologies, USA).[37]

Biomass preparation: *Aspergillus terreus* MTCC 479 was used for comparative analysis with the isolated strain which was revived using Media 117 (MTCC). For biosorption studies, the isolated strain was inoculated in a 100ml nutrient media. The culture was grown at 30°C with regular shaking at 120-150rpm. The biomass obtained after five days of incubation was used for further biosorption studies.

SEM-EDX analysis: The structure of the surface of the chromium loaded biomass and without chromium biomass was analyzed by Carl Zeiss Evo-50 with EDS- Oxford Instruments Nano Analysis**.** The chromium loaded biomass was prepared after incubation of the known and isolated strain *Aspergillus terreus* and *Aspergillus proliferansLA* respectively with Cr(VI) solution of 50ppm concentration. After incubation, chromium loaded cell samples were collected and centrifuged at 13000rpm for 5min, the supernatant was discarded and the pellet was freeze dried at -40°C. The dried samples obtained were covered with gold using a sputter coater and observed at 15kV.

The EDX (Energy Dispersive X-ray analysis) was then performed for the X-ray spectrum analysis of the hexavalent chromium adsorbed by the microbial cells. Similar analysis was carried out for the control in which hexavalent chromium was absent. The microbial cells collected after centrifugation were freeze dried followed by SEM and EDX analysis.

Biosorption in stirred flask system: The analysis for the removal of hexavalent chromium was carried out in stirred flask system. The concentrations of Cr(VI) in each flask were varied according to the different experimental conditions used. The control flasks for each experimental unit were also analyzed for observing the conditions in the absence of hexavalent chromium. The analysis of the biosorption of chromium (VI) was carried out in four different experiments as follows:

Experimental unit I: In this case, the flasks containing 100ml liquid nutrient media with 50ppm of Cr(VI) concentration were inoculated with 2ml of biomass of isolated species and *Aspergillus terreus* respectively in the log phase. The flasks were then incubated at 30°C along with constant stirring at 140rpm.

At regular time intervals, 10ml of the media was taken out and centrifuged at 13000rpm for 5min. The supernatant was then separated and analyzed for Cr(VI) ions using diphenyl carbazide test. The pellet obtained was dried in the oven at 60°C for 4 h and then weighed.

For the diphenyl carbazide test, 5ml of the sample was taken and filtered through a 0.45µm syringe filter to separate the fungal spores followed by adding 45ml of distilled water. The pH of the solution was then adjusted to 1.0±0.3 using 0.2M H_2SO_4. Then to this solution, add 2ml diphenyl carbazide solution, dilute to 100ml with distilled water and

allow to stand for 10min. The absorbance of the solution was recorded at 540nm.

Experimental Unit II: In this case, Cr(VI) was added gradually, 10ppm was added in the log phase and the rest 40ppm in the log phase whereas the inoculum of both, isolated species and *Aspergillus terreus* was added one time. The flasks were incubated under similar conditions i.e. 30°C along with constant stirring at 140rpm. Similarly, samples were taken at regular time intervals and centrifuged at 13000rpm for 5min. The supernatant was then separated and analyzed for Cr(VI) ions using diphenyl carbazide test. The pellet obtained was dried in the oven at 60°C for 4 h and then weighed.

Experimental Unit III: In this case, dextrose (20g/l) was added in the nutrient medium along with 50ppm Cr(VI) concentration and 2ml of inoculum of isolated species and *Aspergillus terreus* respectively. Similarly, the isolated species was tested for 75ppm and 100ppm using the same media composition. The flasks were then similarly incubated at 30°C along with constant stirring at 140rpm. Similarly, samples were taken at regular time intervals and centrifuged at 13000rpm for 5min. The supernatant was then separated and analyzed for Cr(VI) ions using diphenyl carbazide test. The pellet obtained was dried in the oven at 60°C for 4 h and then weighed.

Experimental Unit IV: In this case along with addition of dextrose, Cr (VI) addition was carried out gradually similarly as in experiment II. The flasks were then incubated and samples were collected and analyzed in the similar way.

Experimental Unit V: In this case, dextrose was added 20g/l in the media followed by increasing Cr(VI) concentration, as in 50ppm it was added in the starting of the experiment along with 5ml inoculum followed by adding 20ppm when a 0-1ppm of Cr(VI) concentration is attained. A total of 130ppm of Cr(VI) solution was provided in the case of isolated species *Aspergillus proliferansLA* and 100ppm in the case of known species *Aspergillus terreus*. The flasks were then incubated followed by sample collection and analysis.[11]

Adsorption isotherm studies: In this study, Langmuir and Freudlich adsorption models were analyzed for experimental unit IV using isolated species *Aspergillus proliferansLA* which showed maximum adsorption. The experimental data obtained was used to calculate the values for amount of chromium adsorbed by the adsorbent (Q_e) using the initial chromium concentration (C_0), chromium concentration at equilibrium (C_e), volume of chromium solution used (V) and mass of adsorbent used (m) using the equation:

$$Q_e = (C_0 - C_e)\, V \frac{1}{m} \quad (1)$$

$$\frac{C_e}{Q_e} = \frac{1}{q_{max}\, b} + \frac{C_e}{q_{max}} \quad (2)$$

A plot was constructed between C_e/Q_e and C_e, the slope and intercept were used as the values of Langmuir constants. b is energy of adsorption and q_{max} is maximum adsorption capacity. The correlation coefficient value (R^2) was used to evaluate the fit of the model.

A comparison was done to analyze the better fit isotherm model for the biosorption study, so Freundlich adsorption isotherm was also constructed. It uses two constants: K_f describes the binding affinity and n shows the affinity between adsorbent and solute. The plot is constructed using the values of log C_e and log q_e.

$$q_e = K_F\, C_e^{\,1/n} \quad (3)$$

Adsorption kinetics: The adsorption kinetics of the chromium biosorption data obtained was analyzed and fitted into the Lagergren rate equation model which states that rate of biosorption is proportional to the unoccupied sites available. The equation used for the analysis is given below:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (4)$$

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \quad (5)$$

In the above equations, q_t is the quantity of Cr(VI) adsorbed at time t, q_e is the quantity of dye adsorbed at equilibrium, k_1 is the rate constant and t is the contact time.

Desorption of chromium: The cells obtained in the pellet were washed with distilled water and centrifuged. The supernatant was then discarded followed by addition of 10ml of 0.8M NaOH. The solution was then incubated for 5min., 30min, 1hr., 24hr., 48hr. and the absorbance of the solution was then measured at 540nm using diphenyl carbazide method. The desorption efficiency (D%) and the capacity of desorption ($q_{e,de}$ mg/g) were calculated using the equations 6 and equation 7. In the equations 6 and 7, D% is the desorption efficiency (%), $q_{e,de}$ is the desorption capacity (mg/g), V_{de} (L) is the volume of desorbent solution, $C_{f,de}$ (mg/g) is the concentration of Cr(VI) in desorbent solution at equilibrium and M_{de} (g) is the biomass of the adsorbent used.

$$D\% = \frac{q_{e,de}}{q_{e,s}} * 100 \quad (6)$$

$$q_{e,de} = \frac{V_{de} * C_{fde}}{M_{de}} \quad (7)$$

Results

Isolation and identification of fungal species: The isolation of fungal species was performed using 20ppm of Cr (VI) concentration and was identified by NCL, Pune as *Aspergillus proliferans* CBS121.45 with 99.9% similarity. This initial concentration of 20ppm in the isolation media aided in the adaptation of the microorganism in hexavalent chromium environment. The isolated strain and the sequence

data are deposited in the general collection of microorganisms of the National Centre of Industrial Microorganisms (NCIM) and can be accessed by *Aspergillus sp. (proliferans)* (LA)/ NCIM-1473.

SEM-EDX analysis: The structure of the surface of chromium loaded biomass and without chromium biomass was analyzed by Scanning electron microscope (SEM) with gold coating and Energy dispersive X-Ray analysis. SEM analysis of *Aspergillus proliferansLA* and *Aspergillus terreus* without chromium exposure is shown in figure 1(a) and (b) respectively. The images of chromium loaded biomass of *Aspergillus proliferansLA* and *Aspergillus terreus* are shown in the figure (c) and (e) respectively along with EDX mapping shown alongside in the figure (d) and (f) respectively. The EDX maps clearly indicate the presence of Cr on the surface of both *Aspergillus proliferansLA* and *Aspergillus terreus* which validate the biosorption of Cr(VI) ions strongly.

The structures with rough cavities and heterogenous pores were seen in the figures 1(a) and (b) which are important for potential biosorption of hexavalent chromium. The chromium loaded images figures 1(c) and (e) appear to be close, compact and smoother due to the presence of Cr(VI) ions.

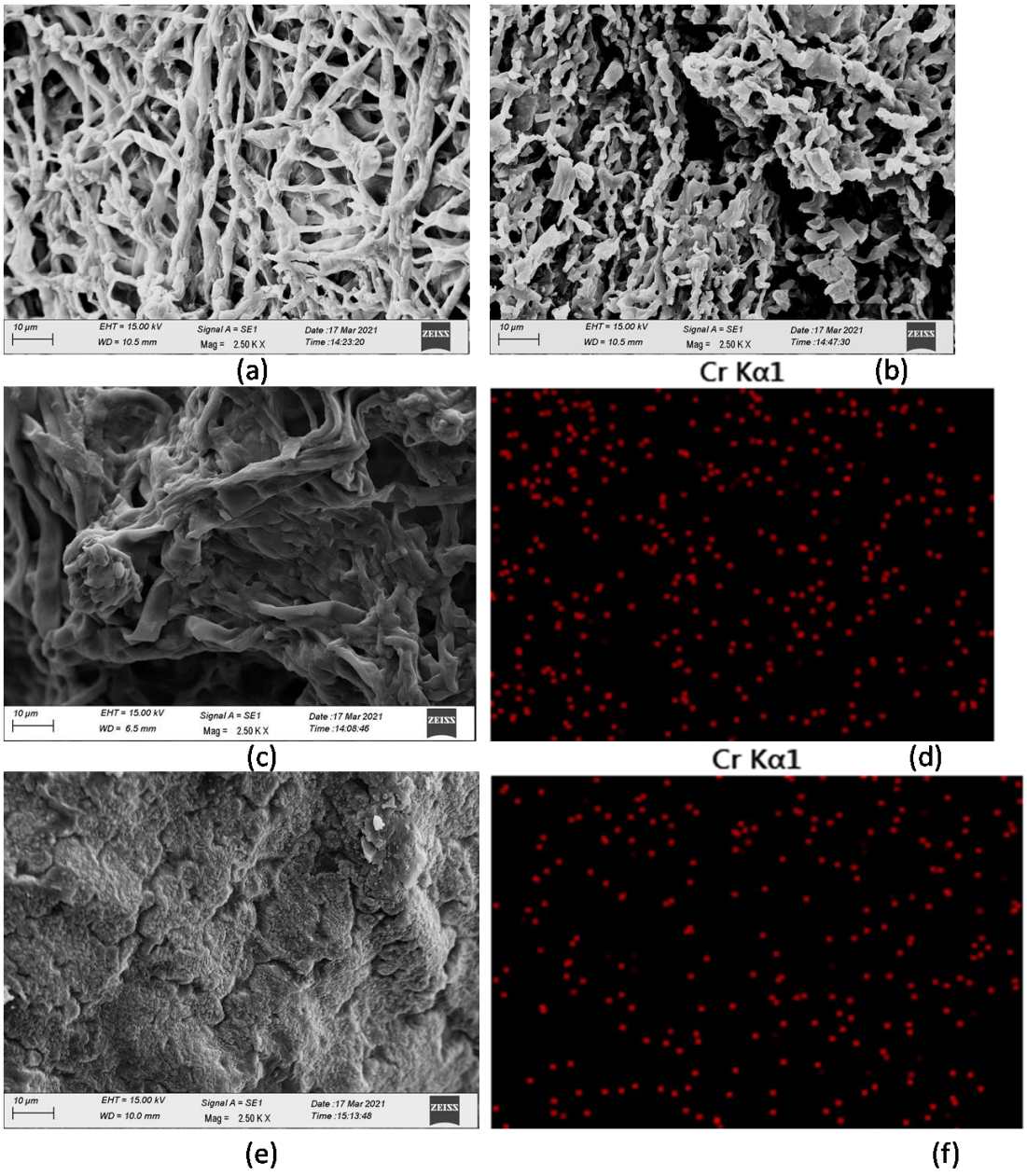


Figure 1: (a) SEM image for isolated strain *Aspergillus proliferansLA* without chromium (b) SEM image for known strain *Aspergillus terreus* without chromium (c) SEM image for isolated strain *Aspergillus proliferansLA* with adsorbed chromium (d) EDX analysis showing Cr(VI) mapped on the surface of *Aspergillus proliferansLA* (e) SEM image for known strain *Aspergillus terreus* with chromium (f) EDX analysis showing Cr(VI) mapped on the surface of *Aspergillus terreus*

Biosorption in stirred flask system: The removal of Cr(VI) with the conc. of 50ppm, 75ppm, 100ppm was compared for the isolated species (*Aspergillus proliferansLA*) and a known species (*Aspergillus terreus*) in different experimental units:

Experimental Unit I: The maximum removal of 82.62% was observed when 50ppm Cr(VI) was added in the lag phase, by using the isolated species *Aspergillus proliferansLA.* 18.94% removal was observed by using the known species *Aspergillus terreus*. The removal observed is shown in the figure 2(a).

Experimental unit II: The maximum removal of 86.97%, was observed when the Cr(VI) of 50ppm conc. was added gradually i.e. Cr(VI) solution of 10ppm conc. was added every 24 h by using isolated species *Aspergillus proliferansLA* as the biosorbent and 63.02% removal was seen when the known species *Aspergillus terreus* was used as the biosorbent. The percentage removal with increasing incubation time is shown in figure 2(b).

Experimental unit III: The metal removal increased to 99.19% using the isolated species *Aspergillus proliferansLA* and 78.43% using known species *Aspergillus terreus* by using dextrose(20g/l) as an additional constituent in the nutrient media being used for the process of biosorption along with one time addition of 50ppm Cr(VI) in the lag phase. Figure 2(c) is showing the percentage metal removal with increased incubation time. A decrease in metal removal was seen when the concentration of Cr(VI) was increased to 75ppm and 100ppm using the same experimental conditions. The metal removal of 72.01% and 71.17% respectively was observed for 75ppm and 100ppm. Figure 3(a) showed the removal percentage of Cr(VI) for 75 and 100ppm concentration using the isolated species *Aspergillus proliferansLA.*

Experimental Unit IV: The percentage removal of 99.479% was achieved in 144h in case of gradual addition of 50ppm of Cr(VI) solution (10ppm added every 24 h) and with modified media (dextrose 20g/l) for isolated species *Aspergillus proliferansLA* and in case of known species *Aspergillus terreus*, removal of 79.166% was achieved in 144h in same experimental conditions. The data for percentage removal for both isolated and known species is given in figure 2(d).

Experimental Unit V: In this case, a total of 130ppm of Cr(VI) was added gradually along with 5ml inoculums of which 86.979% was adsorbed by *Aspergillus proliferansLA*. The known species *Aspergillus terreus* could adsorb 29.68% of 100ppm of Cr(VI) added in a similar manner as in *Aspergillus proliferansLA*. Figure 3(b) shows % removal for 130ppm.

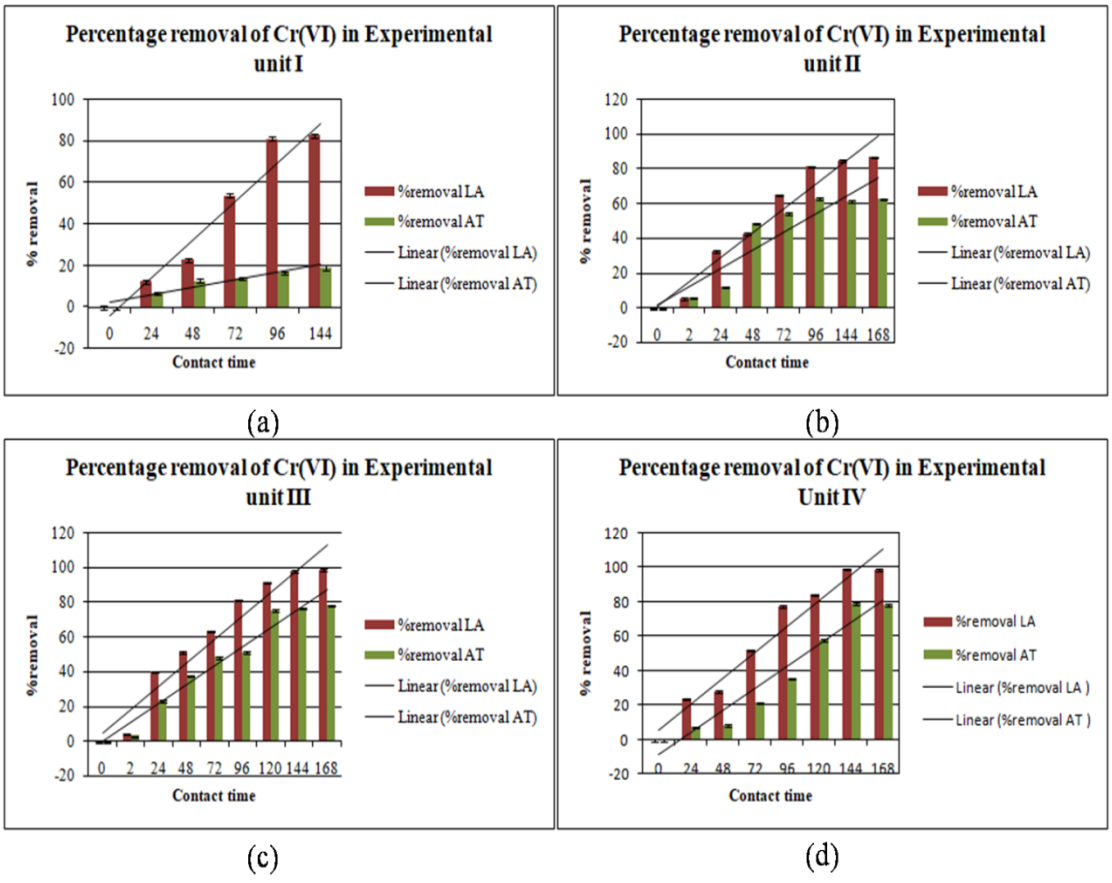
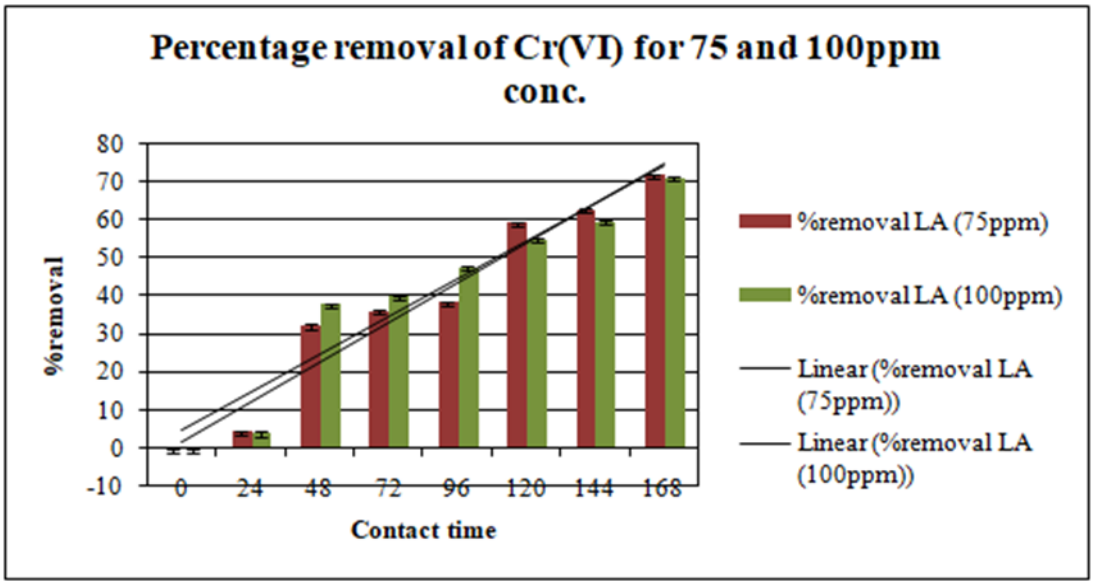
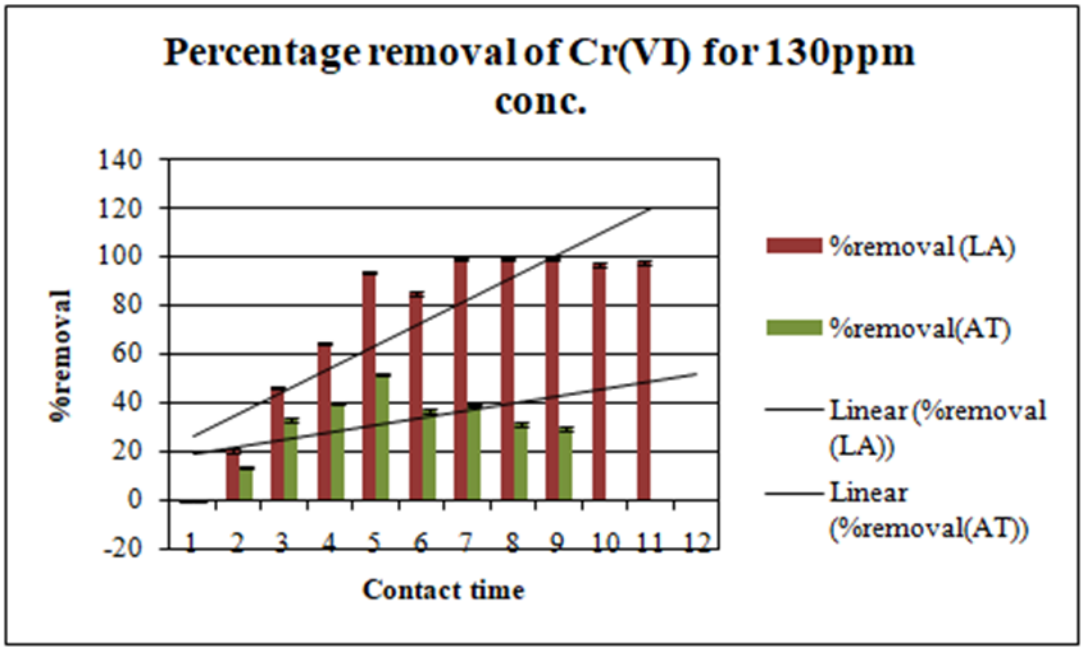


Figure 2: (a) Percentage removal of Cr(VI) using Experimental unit I. (b) Percentage removal of Cr(VI) using Experimental unit II. (c) Percentage removal of Cr(VI) using Experimental unit III. (d) Percentage removal of Cr(VI) using Experimental Unit IV



(a)



(b)

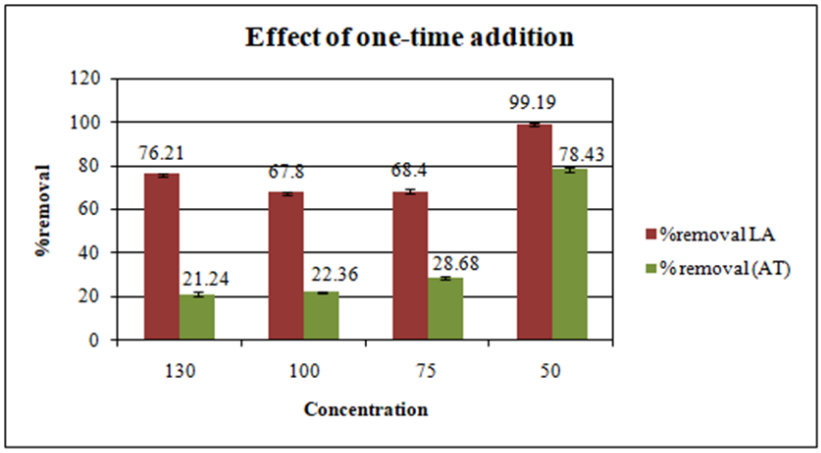
Figure 3: (a) Effect of increasing concentration to 75 and 100ppm on %removal of Cr(VI). (b) Effect of increasing concentration to 130 ppm on %removal of Cr(VI)

Effect of gradual addition of Cr(VI) soln. on removal efficiency: The initial concentration of the adsorbate majorly affects the rate of adsorption, so it is an important phenomenon to be considered for analyzing good adsorption. The major effect of initial concentration of hexavalent chromium is due to growth inhibitory effect on microbes due to the toxic nature of hexavalent chromium.

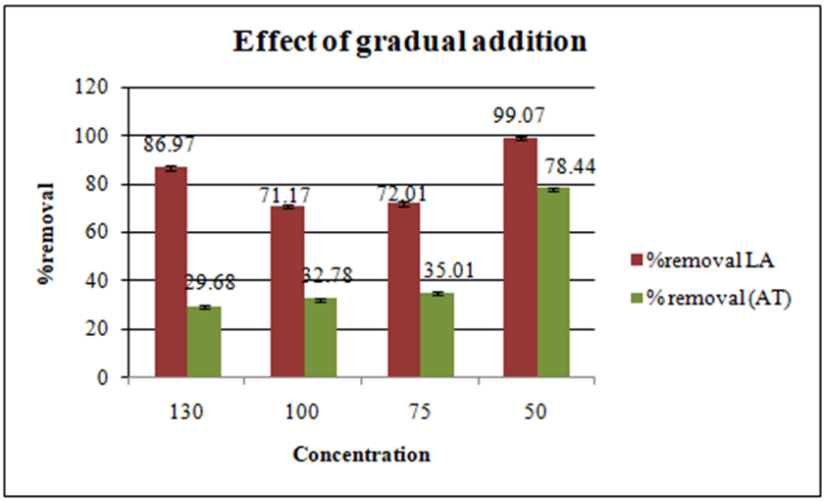
The efficiency of removal when the hexavalent chromium was added gradually to the liquid media, was better than the efficiency obtained in one-time addition of Cr(VI). On one time addition of 100ppm, adsorption increased to 71.17% from 54.02% in gradual addition. Similarly, on gradual addition of 75ppm, adsorption increased to 72.01% from 60.12%. Figures 4(a) and (b) show a comparative analysis for *Aspergillus terreus* and *Aspergillus proliferansLA* in the case of one-time addition and gradual addition of Cr(VI).

Effect of biosorbent dosage: The effect of biosorbent dosages to the sorption media of Cr (VI) with 50ppm conc. was studied at 144h of incubation. The biosorbent doses were varied from 0.10g/l, 0.15g/l, 0.20g/l, 0.25g/l and 0.33g/l. It was inferred that the optimum removal of hexavalent chromium was attained at 0.15g/l, the removal remained constant for biosorbent doses of 0.25g/l and 0.33g/l.

The adsorption of hexavalent chromium ion increases with increase in the biosorbent dosage as the adsorption sites increase with increase in biosorbent dosage. The dosage of 0.33g/l could adsorb 86.97% of 130ppm of Cr (VI) whereas lower efficiency was observed for 0.10g/l and 0.15g/l for 130ppm Cr(VI) soln. The dosage of 0.15g/l could adsorb 99.19% of 50ppm Cr(VI) soln. and the efficiency remained unchanged for 0.33g/l on 50ppm Cr(VI) soln. The effect of biosorbent dosage is shown in figure 5.



(a)



(b)

Figure 4: (a) Effect of one-time addition on % removal. (b) Effect of gradual addition on % removal

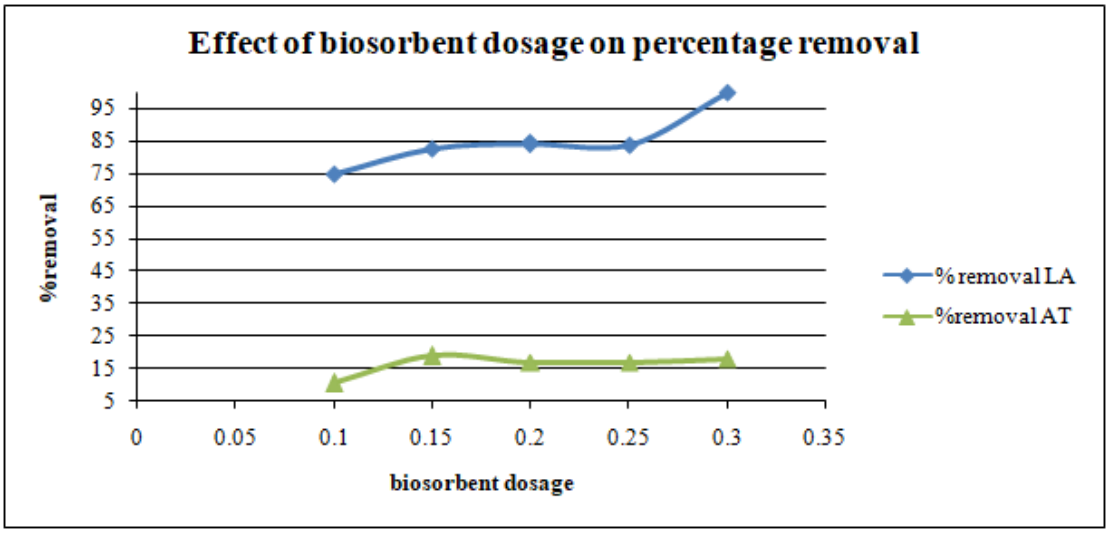


Figure 5: Effect of biosorbent dosage on % removal`

Effect of contact time: Figure 6 shows the uptake of Cr(VI) as a function of contact time. The adsorption of chromium increases as the contact time is increased and becomes constant once the equilibrium is reached. The equilibrium was attained at 144h of incubation for 50ppm of Cr(VI) concentration. Hence, for our study, 144h was chosen as the equilibrium time of incubation.

In a study by Dias et al,[13] on immobilization of *Aspergillus terreus* strain on polyurethane matrix for removal of heavy metals, 96.5mg g^{-1} of chromium was removed from the effluent generated by the steel industry of Brazil. Sriharsha et al[41] studied the absorption and reduction of chromium using *Aspergillus niger* screened on PDA with 5mM of Cr(VI). *A.niger* was able to absorb 98.9% of Cr from 1mM solution of Cr, which further reduced to 29% on increasing the metal concentration to 10mM.[13]

In a study by Srivastava and Thakur,[42] more than 75% removal of chromium was seen by *Aspergillus niger* at pH 6 and temperature 30°C.

Kumaran et al[25] analyzed the adsorption capacity of *Aspergillus terreus* at five different temperatures, different incubation time, different initial Cr(VI) concentration and different initial biomass conc. The dead biomass of *Aspergillus terreus* showed maximum biosorption of 54% at pH 1 and 24h of incubation time and optimum temperature of 27°C, an increase in biosorbent dosage from 1 to 10mg/l increased the biosorbent rate.

Adsorption isotherm studies: The langmuir isotherm was constructed using the values of C_e/Q_e versus C_e in figure 9 where, Q_e is the amount of chromium adsorbed by the adsorbent using the initial chromium concentration C_0, chromium concentration at equilibrium C_e. The values of Langmuir constants and energy of adsorption (b) were calculated as 2.847 and maximum adsorption capacity (q_{max}) as 0.518. The value of correlation coefficient (R^2) is a very important factor of Langmuir isotherm. It indicates the adsorption nature as unfavourable when value is $R^2>1$. It is favourable when $0<R^2<1$. The value of R^2 was calculated as 0.995.

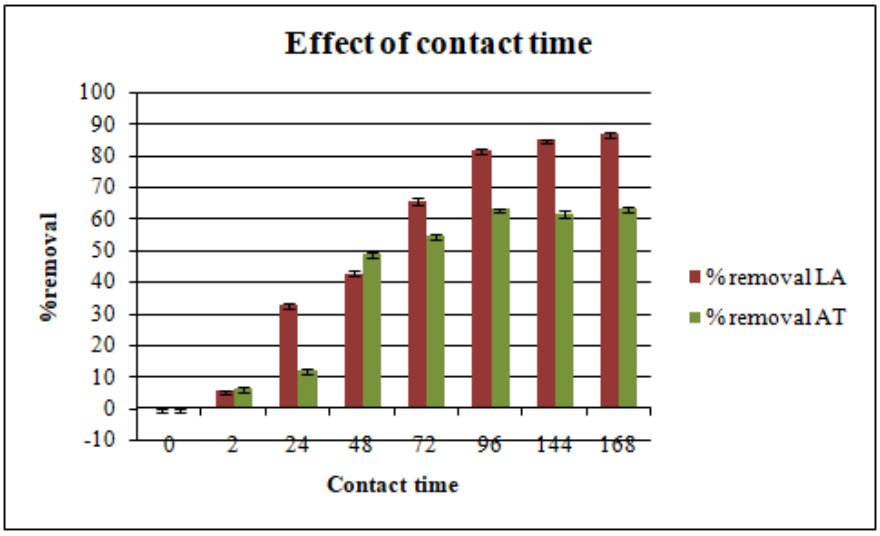


Figure 6: Effect of contact time on %removal

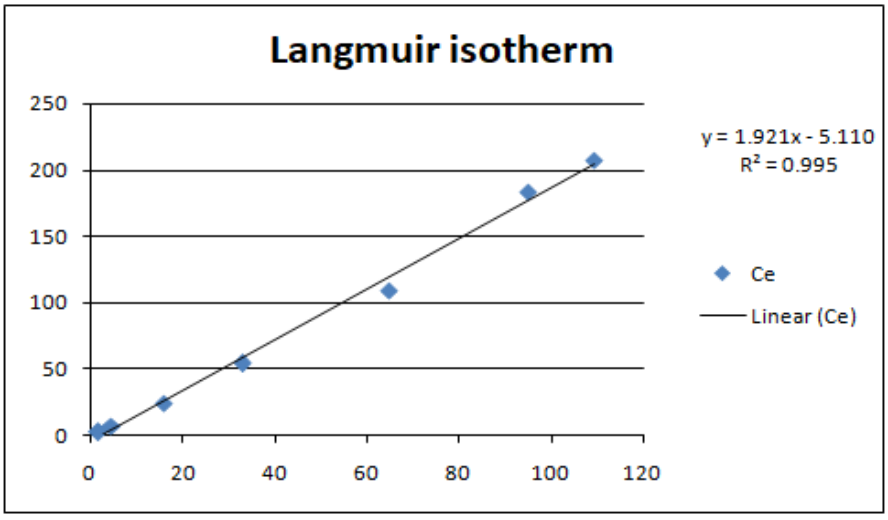


Figure 7: Langmuir adsorption isotherm for adsorption of Cr(VI) on cells of *Aspergillus proliferansLA* in media modified unit (Experimental unit III)

The Freundlich isotherm gave a correlation coefficient (r^2) value of 0.813. The Freundlich isotherm was constructed using the values of log C_e and log Q_e. Figure 10 shows the isotherm.

Adsorption kinetics: The adsorption kinetics of the removal of hexavalent chromium was evaluated by fitting the experimental data obtained and the existing Pseudo-first order kinetic models. The plot was constructed for 50ppm Cr(VI) conc. for different time intervals 24h, 48h, 72h, 96h, 120h, 144h.

Desorption: 0.8M NaOH was used for the process of desorption followed by incubation at different time intervals 5min, 30 min, 1h, 24h and 48h.

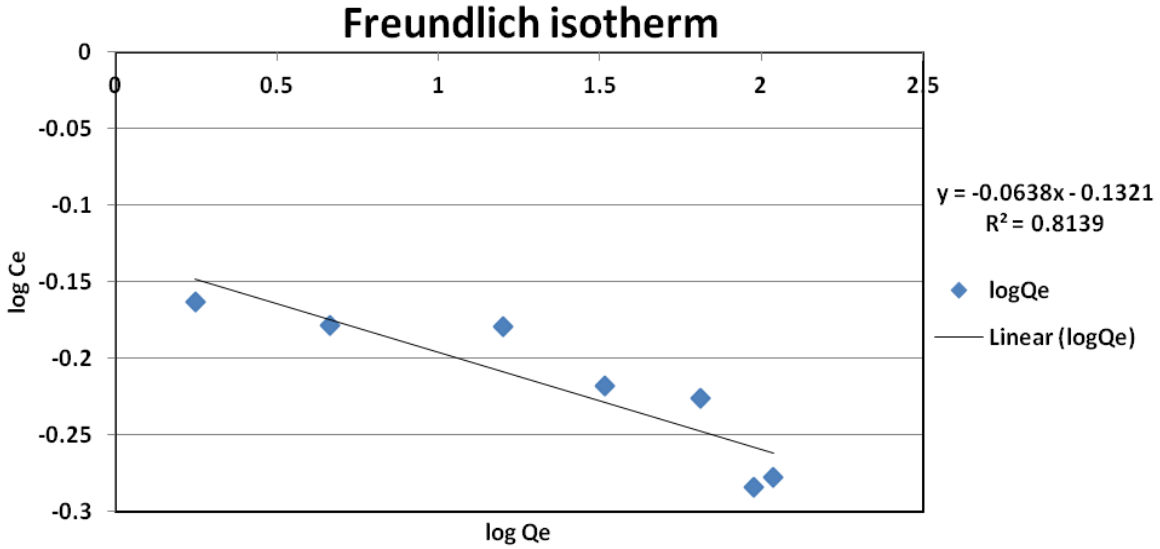


Figure 8: Freundlich adsorption isotherm for adsorption of Cr(VI) on cells of *AspergillusproliferansLA* in media modified unit (Experimental unit III)

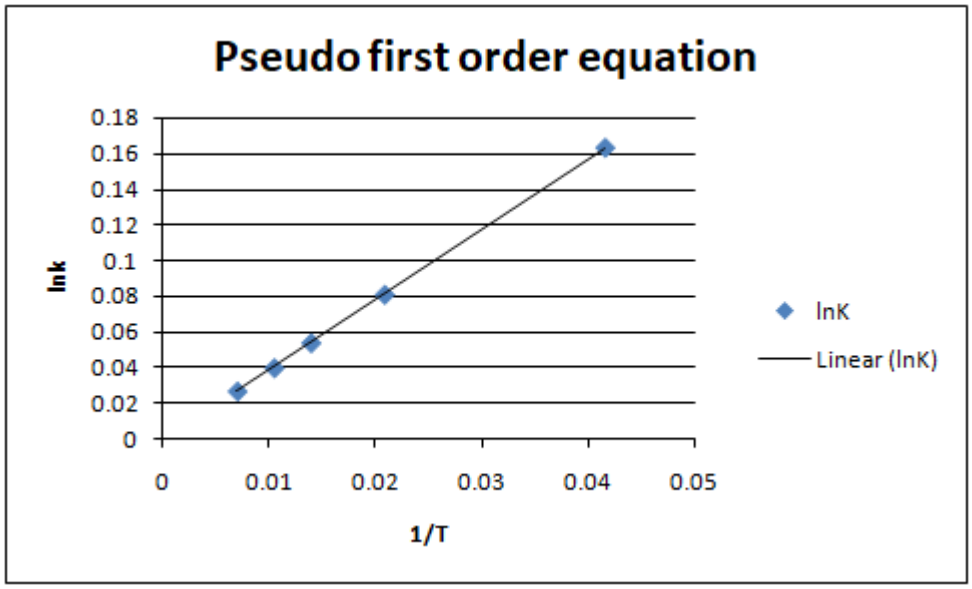


Figure 9: Pseudo-first order equation for biosorption of Cr(VI) with 50ppm conc. on *Aspergillus proliferans* LA biomass

Table 2
Desorption of chromium from the cells of *Aspergillus proliferans* LA

Incubation time	Absorbance	Desorption capacity(%)
5 min	0.005	2.7
30min	0.018	9.72
60min	0.042	22.7
1440min	0.093	50.26
2880min	0.139	75.12

Table 3
Comparative table showing sorption capacity of different forms of biosorbents

S.N.	Biosorbent material	Type of biomass	Metal	Sorption capacity	Isotherm study
1.	*Aspergillus proliferans* LA*	Live	Cr	99.19%	Langmuir R^2= 0.995 Freundlich R^2= 0.813
2.	*Aspergillus terreus**	Live	Cr	78.43%	-
3.	*Cystoseira tamariscifolia*[7]	Dead	Cr	81.96 mg/g	Langmuir R^2= 0.98 Freundlich R^2= 0.79
4.	Sugarcane bagasse[33]		Cr Ni	94% 97.90%	-
5.	*Sargassum oligocystum*[14]	$CaCl_2$ modified	Cr	34.36 mg/g	Langmuir R^2= 0.97 Freundlich R^2= 0.992
6.	*Sargassum oligocystum*[14]	Without modification	Cr	21.57 mg/g	-
7.	*Penicilliumchrysogenum* XJ-1[51]	Live	Cr Cu	52.69±1.68 mg/g 42.83±0.57 mg/g	-
8.	*Kocuria sp.*[2]	Live Dead	Cr	82.4% 69.2%	Langmuir R^2= 0.996 Freundlich R^2= 0.984
9.	*Nostoc sp.*[48]	Live	Cr	20.0 mg/g	Langmuir R^2= 0.991 Freundlich R^2= 0.963
10.	*Aspergillus niger*[40]	Live	Cr	82.1-92.5%	-
11.	*Aspergillus flavus*[40]	Live	Cr	80.6-89.8%	-
12.	*Aspergillus fumigatus*[40]	Live	Cr	79.8-85.4%	-
13.	*Aspergillus nidulans*[40]	Live	Cr	77.8-81.6%	-
14.	*Aspergillus heteromorphus*[40]	Live	Cr	72.4-76.3%	-
15. A	*Aspergillus foetidus*[40]	Live	Cr	68.8-72.6%	-
16.	*Aspergillus viridinutans*[40]	Live	Cr	64.3-67.8%	-
17.	*Rhizopus arrhizus*[35]	HNO_3 treated	Cr	31.52 mg/g	-
18.	*Rhizopus arrhizus*[35]	Dead	Cr	21.72 mg/g	-

***Present Study.**

The maximum desorption of 75.12% was observed when 10ml of 0.8M NaOH was put in metal loaded fungal biomass followed by 48h of incubation. The table 3 shows the desorption capacity with increasing incubation time. No

further improvement in desorption of Cr (VI) was observed after 2880 min. This shows that alkaline desorbing agents favor desorption of hexavalent chromium form the cells of the fungus.

Discussion

The choice of the metal to be adsorbed can be determined by its impact on health and environment and the concentration in which it is present. We chose hexavalent chromium due to its presence in large amounts in the wastewater discharged from the tanneries and textile industries as chromium is used in making chrome salts which are used for tanning the leather; it is also used in certain dyes in major quantity. The isolated strain *Aspergillus proliferansLA* gave high tolerance result with 130ppm of Cr (VI) conc. and gave 86.97% of removal relative to the known strain *Aspergillus terreus* MTCC 479 which could only tolerate 50ppm of Cr(VI) conc. and gave 78.4% of removal.

A similar study was conducted using *Kocuria* sp. by Akbarpour et al[2] in which a comparison was made for removal efficiency using both live and dead forms of *Kocuria* cells. The initial chromium concentration of 25mg/l i.e. 25ppm was used. The live form of cells showed removal efficiency of 82.4% and the dead form showed 69.2% removal efficiency. In this case, Langmuir isotherm model showed better fit than Freundlich adsorption isotherm. The regression coefficient values for Langmuir adsorption isotherm were 0.996 and 0.999 for live and dead form respectively whereas with Freundlich isotherm, regression coefficient values were 0.984 and 0.994 respectively.[2]

The pH plays a major role in the biosorption efficiency of hexavalent chromium. At lower pH, the binding of the metal of interest is reduced as high amount of H^+ ions competes with metal ions whereas with increasing pH, more metal binding is seen as more negatively charged ions are exposed on the biosorbent surface. Warjri et al[48] analyzed the biosorption parameters, isotherm and thermodynamics of the *Nostoc* sp. Seven concentrations of hexavalent chromium were analyzed varying from 1-50 ppm, of which maximum biosorption of 70% was seen at 10ppm Cr(VI) concentration at pH 6.0. The Langmuir isotherm model showed best fit with r^2 value of 0.991 and Freundlich gave r^2 value of 0.963.[48]

In comparison to 99.19% biosorption efficiency at 49.94mg/l Cr(VI) conc. by isolated strain *Aspergillus proliferansLA*, *Aspergillus niger* isolated from the chromium contaminated soils of Nagalkeni, Chennai showed maximum biosorption efficiency of 96.3% at 18.12mg/l Cr(VI) concentration. In our study on isolated strain of *Aspergillus proliferansLA*, no reduction of Cr(VI) was seen i.e. the Cr(VI) adsorbed from the solution was desorbed in the same form with desorption capacity of 75.12% whereas in many studies, some species of *Aspergillus* reduced the hexavalent form of chromium to Cr(III), *Aspergillus flavus* SFL and A1120 reduced the Cr(VI) to Cr(III). The isolated strain *Aspergillus flavus* SFL showed complete reduction at 50 and 100mg/l of Cr(VI) concentration.[46]

Conclusion

The potential of isolated strain *Aspergillus proliferansLA* submitted in NCIM with accession no. NCIM 1473 and a known strain *Aspergillus terreus* MTCC 479 as a biosorbent for Cr(VI) removal from wastewater were analyzed and compared. The characteristics of the biosorbent before and after biosorption were analyzed using SEM-EDX technique which showed possible difference in the images generated and the EDX maps showed presence of chromium. The chromium tolerance analysis of the biosorbent showed *Aspergillus proliferansLA* NCIM 1473 as a potent biosorbent, as maximum growth of 12.86mg/g was obtained at 100ppm Cr(VI) conc. after 120h of incubation whereas for the known strain *Aspergillus terreus* MTCC 479,a maximum growth of 7.06mg/g was obtained after 72h of incubation.

The hexavalent chromium metal biosorption was strongly affected by the initial metal ion concentration and media constituents provided during the biosorption study. The live fungal cells of isolated microorganism *Aspergillus proliferansLA* gave high removal efficiency of 86.97% of hexavalent chromium with 130ppm concentration whereas the known species *Aspergillus terreus* could adsorb 79.16% of hexavalent chromium with 50ppm conc. The adsorbed hexavalent chromium was desorbed using 0.8M NaOH with desorption efficiency of 75.12%. The removal efficiency decreased with increase in Cr(VI) concentration of the solution.

Hereby, considering the potential of the biosorbent, it can be concluded that the biomass of *Aspergillus proliferansLA* NCIM 1473(with media modification) can be used as a suitable biosorbent for removal and recovery of hexavalent chromium from the industrial wastewater. The Cr(VI) adsorbed by the biomass can be recovered by desorption and the biomass can be reused for biosorption of Cr(VI).

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