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Hexavalent chromium biosorption by dried biomass of *Aspergillus niger* NUA101 isolated from Indian ultramafic complex

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Mining operations in Sukinda and Baula-Nuasahi region of Orissa, India have led to the generation of a huge amount of overburden with considerable amount of chromium. Weathering of chromium by the natural agencies often contaminates the water sources of the region. Microbes indigenous to this environment having the potential of accumulating metals could be employed for removal as well as detoxification of heavy metals. Chromium resistant fungi were isolated and purified from the chromite mining environment and evaluated for their potential to biosorb hexavalent chromium using dried fungal biomass. *Aspergillus niger* NUA101, the best sorbent removed about 17.58 mg Cr(VI)/g biomass from a solution containing 25 mg Cr(VI)/l. The sorption capacity of the isolate was standardized following Langmuir and Freundlich adsorption isotherm models and the process was optimized for several standard parameters like, initial metal ion concentration, sorbet concentration, incubation temperature, pH, presence of additional cations and chemical treatment of the biomass. Under optimized conditions, *A. niger* mycellial mass appeared to be effective in removing Cr(VI) from aqueous solution.

Key words: Hexavalent chromium, fungal biosorption, *Aspergillus niger* NUA101, chromite mining overburden, heavy metal detoxification.

INTRODUCTION

Chromium is one of the world's most critical and highly soluble metal pollutants having diverse use in several industries such as stainless steel and non-iron alloy production for metal plating, pigment development, metal processing, catalyst production, surface treatment and in refractories. In the environment, chromium exists in two stable forms, the hexavalent chromium [Cr(VI)] and the trivalent one [Cr(III)]. The hexavalent chromium is highly toxic and carcinogenic in nature and is a potential contaminant in soil, surface water and ground water (Mishra et al., 2009). Environmental pollution caused by chromium and its compounds is mainly because of large number of industrial operations including mining, chrome plating, pigment production, petroleum refining, leather tanning, wood preserving, textile manufacturing, pulp processing and electroplating (Barlett and James, 1988).

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Detoxification of Cr (VI) produced from the mine tailings particularly from chromite mines is an essential approach from environmental point of view. The conventional physico-chemical processes of metal detoxification are very expensive and not environment friendly. Therefore, metal binding capacity of several microbes has been explored to decontaminate polluted aquatic sources in an eco-friendly as well as cost-effective way (Das et al., 2008). Several microorganisms including bacteria, algae, yeast and fungi have been reported to effectively accumulate heavy metals both in living and dead forms (Jia et al., 2014; Conjeevaram et al., 2007; Mala et al., 2006). However, inert substances like cork are no exception for Cr(VI) adsorption from industrial waste water (Sfaksi et al., 2014). Removal of Cr(VI) from the untreated tannery effluents around East Kolkata Wetlands by *Pseudomonas aeruginosa* has been studied (Chatterjee et al., 2011). Likewise, Cr(VI) biosorption and bioaccumulation by *Morganella morganii* has been documented by Ulger et al. (2014) and studies on removal of Cr(VI) and Ni(II) by heavy metal resistant *Aspergillus niger* and *Micrococcus* sp. isolated from the soil of an electroplating industry (Congeevaram et al., 2007) has been established.

The solid microbial biomass, due to its higher affinity for the dissolved metal species attracts complexes and removes it from the aqueous phase by different mechanisms. The process continues till equilibrium is established between the amount of solid bound sorbet species and its portion remaining in the solution (Das et al., 2008).

Fungal biomaterials due to their inherent physico-chemical properties have been established as efficient biosorbents. High percentage of the cell wall material and availability of fungal biomass as a by-product of various antibiotic and food industries makes it an obvious choice as suitable biosorbent (Das et al., 2008). Removal of Cd(II) and Pb(II) has been reported by fungal species in batch and continuous reactors (Huang et al., 1994) and by immobilized *Rhizopus nigricans* (Kogrej and Pavko, 2001). Similarly, Pal et al. (2006) reported the biosorption of cobalt by *Morteriella* sp. isolated from the serpentine soils of Andaman. Srivastava and Thakur (2006) reported the use of *A. niger* as biosorbent to remove Cr(VI) from tannery effluent, while, Kovasevic et al. (2000) documented the biosorption of chromium, copper, nickel and zinc by fungal pellets of *A. niger* 405. Uptake of chromium by living cells of *A. foetidus* (Prasenjit and Sumathi, 2005) and remediation of Cr(VI) contamination from waste water by *A. flavus* is not uncommon (Deepa et al., 2006). Biosorption of Ni(II) and Pb(II) as well as Cr(VI) and Fe(III) from binary metal solutions by *Phanerochaete chrysosporium* (Ceribasi and Yetis, 2001; Marandi, 2011) and *R. arrhizus* respectively (Sag and Kutsal, 1996) has also been well established.

Mining overburden and mine tailings are rich in heavy metals due to their origin, and the microorganisms that flourish in these heavy metal containing areas obviously exhibit metal tolerance as their integral property and hence can be effectively utilized in decontamination of metal polluted sites (Mishra et al., 2009). Reports of metal tolerant microbes indigenous to geologically rich areas are not uncommon (Bohidar et al., 2009; Pal et al., 2005; Mulligan et al., 2004; Rao et al., 2002; Castro et al., 2000). Although literature pertaining to the isolation of chromium resistant fungi from chromite mining environment are scanty, *Aspergillus* and *Penicillium* spp. resistant to chromium have been isolated from chromium deposits of Fukuoka, Japan and evaluated for the abilities to remove Cr(VI) from the contaminated soil (Fukuda et al., 2008). The chromite mines of Orissa, India also harbor some chromium tolerant isolates both bacteria and fungi (Das et al., 2013; Samuel et al., 2012; Behra et al., 2010; Gupta et al., 2007) that has been employed both for metal prospecting and remediation.

The chromite mines of Sukinda and Baula-Nuasahi belt of Jajpur and Keonjhar districts of Orissa, India, respectively form a part of the famous chromite bearing Sukinda ultramafic complex. Mining is conducted by both open-cast and underground techniques in this area leading to the generation of a huge amount of overburden, nearly 8 to 10 times of that of the ore (Acharya et al., 1998), which also represent huge reserve of nickel (0.99%), chromium (2.59%), iron (48.8%) and cobalt (0.03%) (Behra et al., 2011). Overburden generated through the process of mining is dumped in and around the mining site in huge piles ranging from 3 to 10 m in height (Rath et al., 2010). This huge amount of solid waste is subjected to continuous environmental weathering resulting in lowering of the water table as well as, deterioration of surface and ground water quality (Mishra and Sahu, 2013). In this region, the ground water is reported to be polluted by 0.03-0.08 mg/l of Cr(VI) (Tiwary et al., 2005), which demand effective bioremediation measures for detoxification as well as removal of hexavalent chromium.

Therefore, in the present communication attempts have been made to isolate chromium resistant fungi from the chromite mining overburden of Sukinda and Baula-Nuasahi regions of Orissa, India, and optimize the conditions using the dried fungal biomass as biosorbent from aqueous solution for effective removal of hexavalent chromium.

MATERIALS AND METHODS

Isolation of chromium resistant fungi

Chromium resistant fungi were isolated from 22 overburden samples (Cr content 0.02 - 16.56 mg/g) collected from the chromite mining areas of Sukinda and the Baula-Nuasahi regions of Orissa, India. The samples were serially diluted and plated on Czapek-Dox agar supplemented with 50 mg/l of Cr(VI) (K_2CrO_4). Morphologically distinct chromium resistant fungi which appeared on the plates after 5-7 days of incubation at 30°C were purified and the pure cultures

were maintained on slopes of Czapek-Dox agar by sub-culturing at regular intervals.

Evaluation of chromium resistance

In order to evaluate chromate resistance, all the fungal isolates were grown on Czapek-Dox agar plates supplemented with increasing concentrations (2-6 mM) of Cr(VI), and the growth of the isolates were observed over a period of 8 days. Minimum inhibitory concentration (MIC) of Cr(VI) for the resistant fungi was also determined following broth dilution method (Pal et al., 2004). The fungi were allowed to grow at 30°C in 20 ml Czapek-Dox medium/100 ml flask containing 1.0 - 6.0 mM Cr(VI) and the minimum concentration of the metal in the medium completely inhibiting the growth of fungi was considered as the minimum inhibitory concentration (MIC).

Preparation of fungal biomass

Fungal biomass was prepared by growing the fungal isolates in Czapek-Dox medium using shake flask method. Flasks (50 ml/250 ml flask) were inoculated with homogenous spore suspension (10^6 spores/ml) in Tween 80 (0.1% w/v) and incubated on a rotary shaker (120 rpm) for 8 days at 28°C. The biomass was harvested by centrifugation at 10,000 g for 10 min using Remi R24 centrifuge and washed thoroughly in double distilled water. The fungal mycelia thus obtained were dried overnight at 80^0C. The dried mycelial mass were ground in mortar pestle and sieved through 0.1 mm mesh and stored at 4^0C in sterilized containers for further use.

Biosorption studies

The dried fungal biomass (2 g/l) was suspended in 20 ml of Cr(VI) solution (25 mg/l) contained in 100 ml Erlenmeyer flasks. The set-up so prepared was incubated under continuous shaking at 120 rpm on a rotary shaker for 48 h at 30°C. Samples were withdrawn at regular interval, centrifuged at 10,000 *g* for 10 min, the supernatant was filtered through Whatman (No. 42) filter paper and evaluated for the residual Cr(VI).

Estimation of Cr(VI)

The amount of metal biosorbed was determined by measuring the residual Cr(VI) in the supernatant using Diphenylcarbazide (DPC) as the complexing agent (APHA, AWWA, WEF, 1998). To 1 ml of the supernatant, 1 ml of 0.05% diphenylcarbazide solution in acetone and 3 ml of 0.16 M sulfuric acid was added and the absorbency of the reaction mixture was measured spectrophotometrically at 540 nm. Amount of Cr(VI) was determined from the calibration curve prepared in the same way. All experiments were performed in triplicates and results represent mean ± standard deviation.

RESULTS

Evaluation of chromium resistance

In order to obtain potential Cr(VI) tolerant isolates, a total of 119 fungal cultures were isolated from the mining overburden samples collected from different chromite mining sites of Sukinda and Baula-Nuasahi region of Orissa, India. The isolates were then subjected to screening for Cr(VI) tolerance by solid-plate assay on Cr(VI) supplemented (1-6 mM) Czapek-Dox agar. Based on the visual growth of the isolates on solid media, it was observed that 20 out of 119 fungi were efficient in growing in media supplemented with 5 mM Cr(VI). None of the isolates were able to grow on plates containing 6 mM of Cr (VI) (Figure 1).

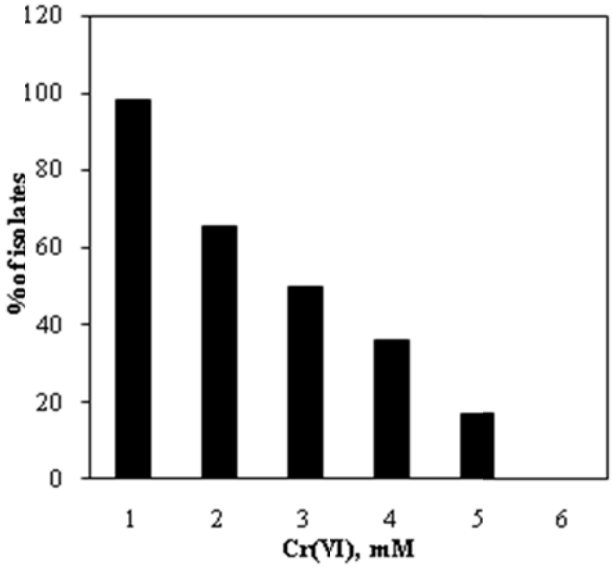

Figure 1. Hexavalent chromium tolerance profile of fungal isolates indigenous to chromite mine overburden sample (Tolerance of isolates was estimated by measuring the growth of the fungi visually after incubation on Czapek Dox agar medium supplemented with 1-6 mM of Cr(VI)).

All 20 fungal isolates that tolerated 5 mM Cr(VI) were further characterized for their micro-morphological features and compared with those described in the Manual of Soil Fungi (Gilmann, 1957), Manual of Penicillia (Raper and Thom, 1984) and Manual of Aspergilli (Thom and Raper, 1946) to determine their taxonomic identity. Species identity of the fungal isolates was further confirmed by National Council for Fungal Taxonomy (NCFT), New Delhi, India. Eight of them belonged to *Aspergillus*, nine strains to *Penicillium*, and one isolate each of *Morteriella, Fusarium* and *Trichoderma*.

Screening for chromium biosorption

The selected Cr (VI) tolerant fungal isolates (20) were further subjected to Cr (VI) biosorption studies using aqueous solution of hexavalent chromium and dried fungal mycellial mass. The dried powdery biomass (2 g/l) was added to the aqueous Cr (VI) solution (25 mg/l) and incubated for 48 h under continuous shaking (120 rpm) at 30°C. It was observed that all the selected isolates were capable of absorbing Cr (VI) ions from the aqueous solution, however, the degree at which metal ions were

Table 1. Screening of dried mycellial biomass of chromium resistant fungal isolates for biosorption of hexavalent chromium.

Isolate	Cr(VI) biosorbed (mg/g)	
	Incubation (h)	
	18	48
Aspergillus niger SAU206	10.25±0.25	11.37±0.37
A. niger SUK101	9.75±0.25	11.50±0.50
A. niger SUK703	14.62±0.37	15.50±0.25
A. niger NUA101	13.33±0.58	17.58±0.08
A. phoenicus SAU207	11.25±0.25	13.50±0.50
A. versicolor SUK403	9.0±0.50	10.87±0.12
A. versicolor SKP206	11.12±0.12	15.25±0.00
A. humicola SKP102	12.25±0.25	15.75±0.00
A. awamori BOU108	11.00±1.00	13.00±0.00
Penicillium sp. SUK107	11.87±0.87	14.25±0.50
Penicillium sp. SKP302	10.50±0.50	12.87±0.12
Penicillium sp. SAU202	9.00±1.00	9.25±1.25
Penicillium sp. SUK701	9.00±0.00	10.37±0.37
Penicillium sp. SUK507	10.12±0.12	15.50±0.25
Penicillium sp. SUK705	12.62±0.62	15.00±0.05
P. citrinum NUA204	12.00±0.50	15.83±0.58
P. simplicissimum SAU203	12.00±0.50	15.00±0.00
Morteriella sp. SUK201	11.12±0.37	12.50±0.00
Fusarium sp. SKP101	8.62±0.37	9.25±0.25
Trichoderma sp. SUK503	9.37±0.37	9.75±0.25

Biosorption studies were conducted in batch mode with an initial biomass and Cr(VI) concentration of 2g/l and 25 mg/l and pH 7.0 respectively. Samples were incubated at 30°C for 48 h under continuous shaking (120 rpm). Results represent means of triplicates ± SD.

removed by the biomass varied significantly (Table 1). Moreover, biosorption of chromium was rapid during the first 18 h of incubation but continued up to 48 h irrespective of the fungal isolates.

After 18 h of metal-biomass interaction, many of the isolates including *A.niger* SUK703, *A. niger* NUA101, *A. humic*ola SKP102, *Penicillium* sp. SUK705, *P. citrinum* NUA204 and *P. simplicissimum* SAU203 exhibited more or less similar range of Cr(VI) biosorption. However, at prolonged incubation (48 h), it appeared that *A. niger* NUA101 could remove 70% of Cr(VI) from the aqueous solution and showed a metal loading capacity of 17.58±0.08 mg Cr(VI)/g biomass.

Similarly, metal loading capacities of a number of *Aspergillus* and *Penicillium* isolates were not inferior, which ranged from 15.0 to 15.83 mg/g of Cr(VI). *A. niger* NUA101, the best sorbent was selected for further studies to determine the optimal conditions for Cr(VI) sorption.

Time course of Cr(VI) biosorption

Time course of Cr(VI) biosorption by the dried mycellial mass of *A. niger* NUA101 showed strong affinity for chromium binding and sorbed considerable quantities of metal [13.33±0.58 mg Cr(VI)/g biomass] within 18 h of incubation. The metal loading capacity of the biomass, however, increased steadily with time and an equilibrium of 17.58±0.08 mg/g Cr(VI) was achieved after 48 h of incubation (Figure 2). Subsequent sorption experiments were, therefore, conducted till 48 h of incubation.

Effect of chromium concentration

The biosorption of Cr(VI) by *A. niger* NUA101 increased with an increase in initial metal ion concentration (Figure 3). It was observed that with an increase of the initial metal ion concentration from 25 mg/l to 100 mg/l, metal sorption increased from 9.04±0.42 mg Cr(VI)/g biomass to 30.0±0.5 mg Cr(VI)/g biomass. There was however, no significant increase in the metal loading with further increase in Cr(VI) concentration up to 150 mg/l.

Biosorption isotherms

Chromium biosorption equilibrium was quantified by

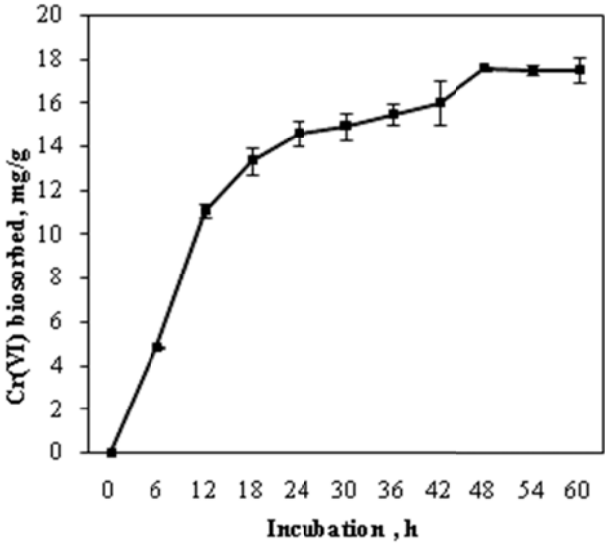

Figure 2. Time course of Cr (VI) biosorption by dried mycelial biomass of A. niger NUA101 [Biosorption studies were conducted in batch mode with an initial biomass density of 2g/L, shaking speed of 120 rpm, incubation temperature of 30°C and pH: 7.0. Initial metal ion concentration was 25 mg/l Cr(VI) and incubation period was 48 h].

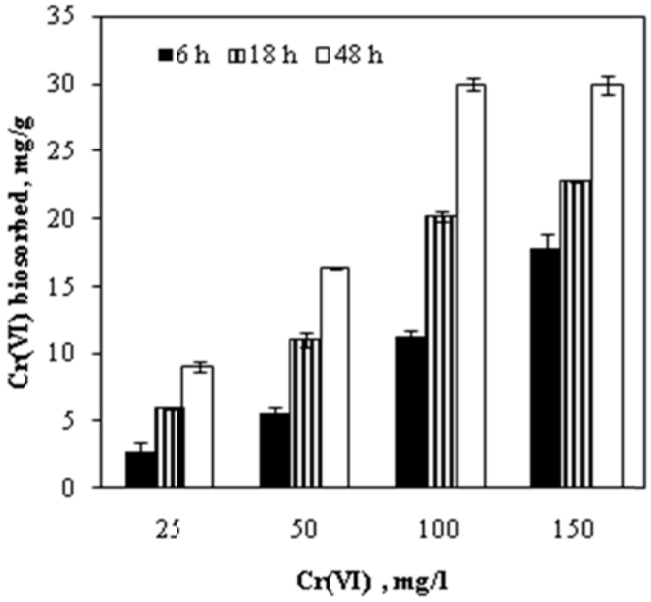

Figure 3. Effect of initial sorbate concentration on Cr (VI) biosorption by *A. niger* NUA101 [Biosorption studies were conducted in batch mode with an initial biomass density of 2 g/L, shaking speed of 120 rpm, incubation temp of 30°C and pH: 7.0. Incubation period was 48 h].

standard Langmuir and Freundlich adsorption isotherms using the following formulae:

Langmuir model: $Q_{eq} = Qmax.(b).C_{eq}/1+(b).C_{eq}$
Freundlich model: $Q_{eq} = K(C_{eq})^{1/n}$

Where, Q_{eq} is the amount of metal ion biosorbed at equilibrium per unit weight of biomass; C_{eq} is the metal ion concentration at equilibrium; Q_{max} and b are Langmuir model constants and K and n are Freundlich model constants.

The linearized Langmuir and Freundlich adsorption isotherms for hexavalent chromium using *A. niger* NUA101 biomass are shown in Figure 4A and 4B, respectively. The adsorption constants were calculated from the respective isotherms along with correlation coefficients (Table 2). The constants for Langmuir model, 'Q_{max}' and 'b' were 138.26 and 0.0017, respectively; while those for Freundlich isotherm, 'K' and 'n' were 1.31 and 1.604.

Effect of biomass concentration

The effect of initial biomass concentration on Cr(VI) biosorption by *A. niger* NUA101 was evaluated using a biomass concentration ranging from 1-5 g/l, while the Cr(VI) concentration was maintained at 25 mg/l. Figure 5 indicates that the metal loading capacity of the biomass was greatly influenced by the initial concentration of dried mycelial mass used. The maximum chromium sorption was achieved with initial biomass concentration of 1 g/l but sorption capacity declined rapidly with increase in biomass level.

Effect of temperature and pH

Biosorption of heavy metal ions from aqueous solution is strongly influenced by the pH and the temperature of the solvent. In *A. niger* NUA101, optimum temperature was recorded at 30°C, where, 17.92±1.17 mg Cr(VI)/g biomass was sorbed in 48 h (Figure 6A). Similarly, optimum pH for Cr(VI) biosorption was 6.0, whereby, 17.08±0.08 mg Cr(VI)/g biomass was sorbed in 48 h (Figure 6B).

Effect of additional ions

Presence of additional cations in the same phase often results in competitive biosorption affecting the metal loading capacity of the biomass. The influence of multivalent cations such as, Ni(II), Co(II), Zn(II) and Fe(III) on biosorption of Cr(VI) by *A. niger* NUA101 biomass was evaluated by supplementing the sorption media at equivalent concentration. Biosorption of Cr(VI) in presence of Ni(II), Zn(II) and Fe(III) was found to be facilitated, while it was more or less unaffected when Co(II) was added (Figure 7). It was observed that under

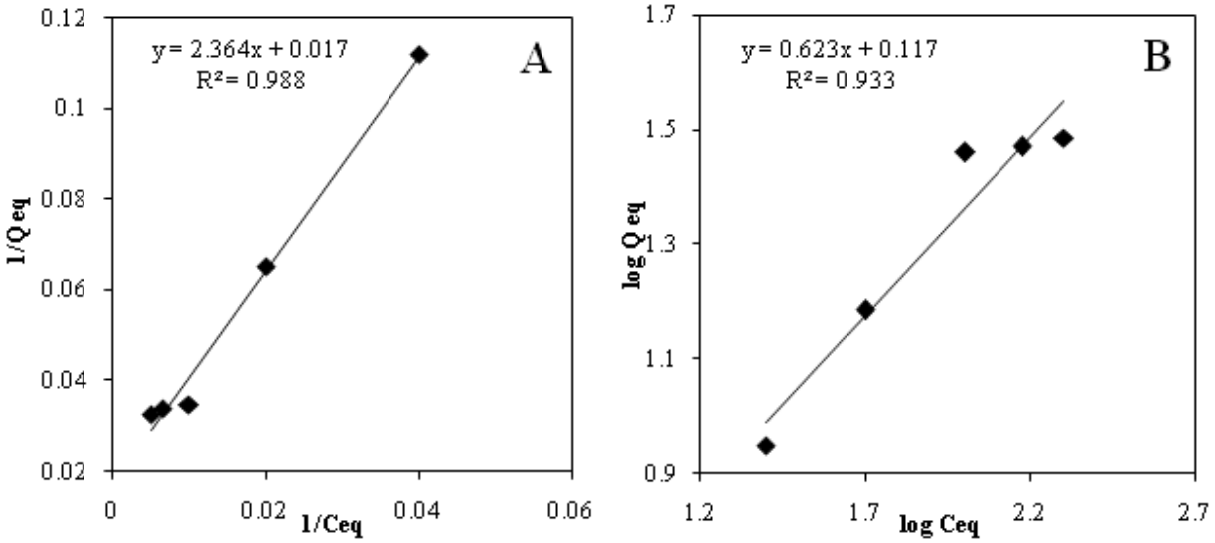

Figure 4. The linearized Langmuir (A) and Freundlich (B) adsorption isotherms of Cr(VI)) by *A. niger* NUA101 biomass.

Table 2. Equilibrium isotherms for Cr(VI) biosorption by mycelia biomass of *A. niger* SUK101.

Model	Equation	Isotherm constants	Correlation coefficient
Langmuir	$Qeq = \frac{2.3643Ceq}{1 + 0.0171Ceq}$	Q_{max}=138.26 b= 0.00171	0.9889
Freundlich	$Q_{eq} = 1.31C_{eq}^{1.14}$	K= 1.31 n= 1.604	0.9332

Q_{eq} is Cr(VI) adsorbed (µg/g biomass); Ceq is residual chromium (µg) at equilibrium.

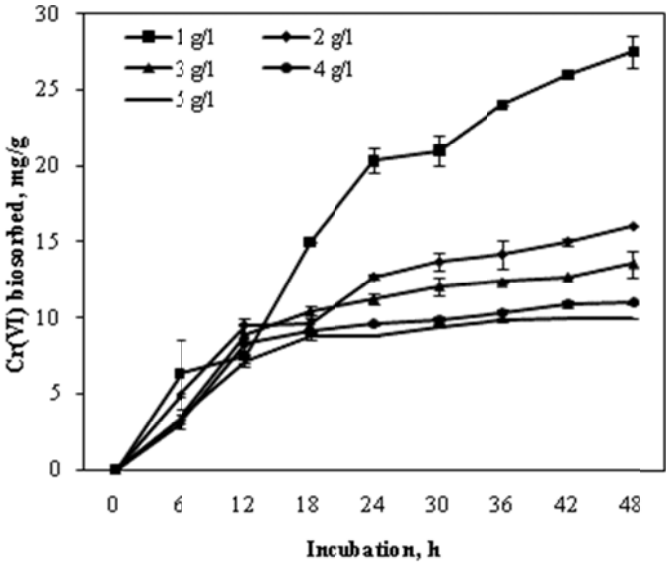

Figure 5. Effect of initial biomass concentration on Cr (VI) biosorption by *A. niger* NUA101 [Biosorption studies were conducted in batch mode with initial metal ion concentration of 25 mg/L Cr(VI) and incubation period was 48 h, shaking speed was 120 rpm, incubation temp and pH were set at 30°C and 7.0, respectively].

control condition, 17.25±0.5 mg Cr(VI)/g biomass was sorbed whereas, with addition of 2 mg/l Ni(II) and Zn(II), Cr(VI) biosorption was found to be increased to 18.83±0.33 and 20.25±0.5 mg Cr(VI)/g biomass respectively. Addition of Fe(III) at equal concentration augmented the sorption process significantly resulting in the uptake of 25.0 mg Cr(VI)/g biomass.

Effect of pre-treatment of biomass

Pre-treatment of biomass with various chemical and physical agents often affect the proficiency of sorption as they alter the metal binding sites of the fungal biomass. Autoclaving the biomass at 15 psi for 15 min as well as treatment of biomass with 0.5 M sulfuric and acetic acid for 30 min reduced the rate of biosorption, whereas, alkali (0.5 M NaOH and Na_2CO_3) treatment enhanced the process (Figure 8). Cr(VI) sorption using untreated fungal biomass was recorded to be 17.25±0.5 mg Cr(VI)/g biomass, whereas, NaOH and Na_2CO_3 treatment resulted in sorption of 19.0±0.11 and 18.25±0.83 mg Cr(VI)/g biomass respectively. Tween 80 (0.5 M) treated biomass resulted in maximum sorption accounting removal of 21.0±0.42 mg Cr(VI)/g biomass in 48 h.

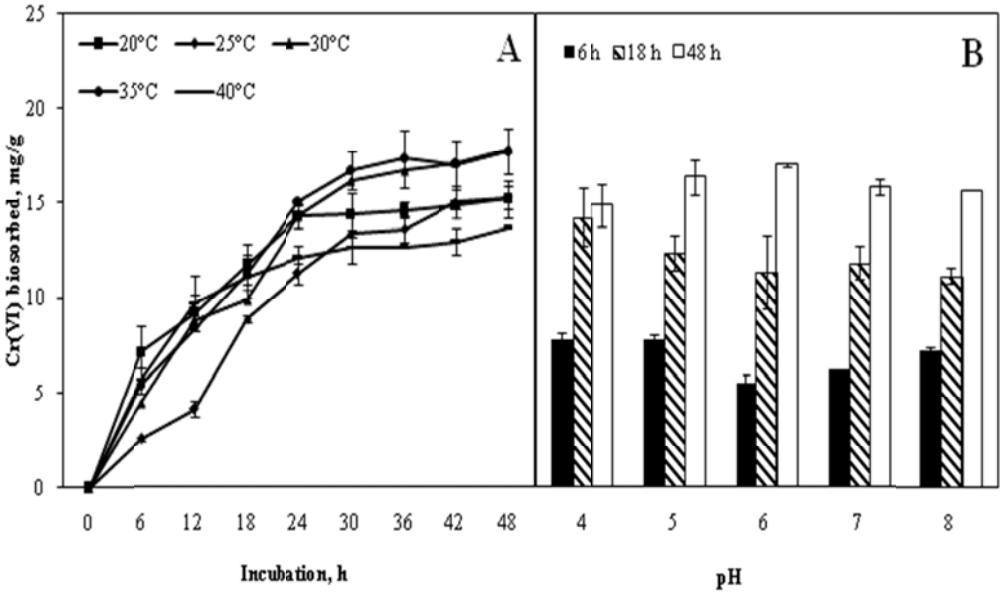

Figure 6. Effect of (A) incubation temperature and (B) initial pH of the reaction mixture on Cr (VI) biosorption by A. niger NUA101 [Biosorption studies were conducted in batch mode with an initial biomass density of 2 g/L and shaking speed of 120 rpm. Initial metal ion concentration was 25 mg/l Cr(VI) and incubation period was 48 h].

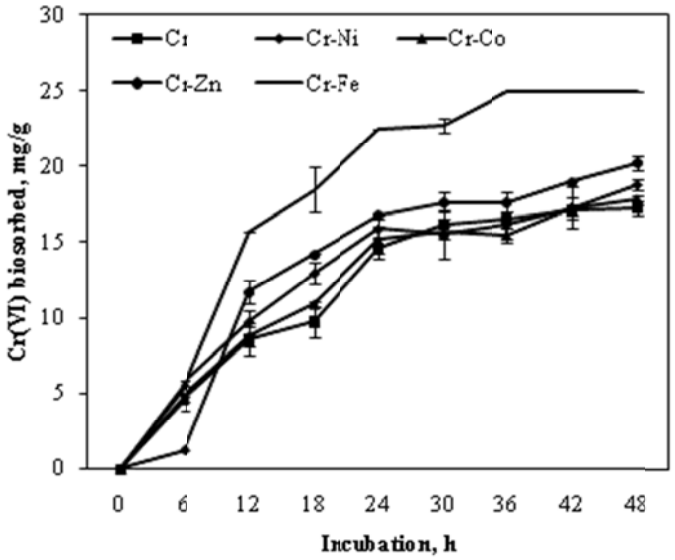

Figure 7. Effect of the presence of additional cations on Cr(VI) biosorption by A. niger NUA101 [Biosorption studies were conducted in batch mode with an initial biomass density of 2 g/L, shaking speed of 120 rpm, incubation temp of 30°C and pH: 7.0. Initial metal ion concentration was 25 mg/l Cr(VI) and incubation period was 48 h].

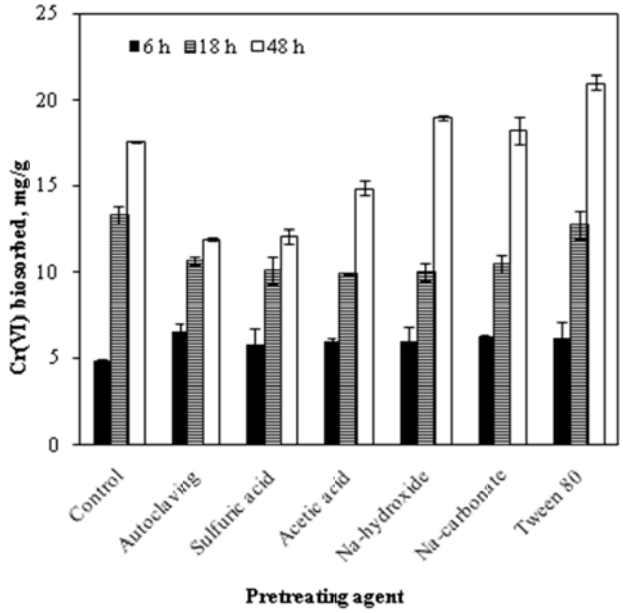

Figure 8. Effect of pre-treatment of biomass on Cr(VI) biosorption by *A. niger* NUA101 [Biosorption studies were conducted in batch mode with an initial biomass density of 2 g/L, shaking speed of 120 rpm, incubation temp of 30°C and pH: 7.0. Initial metal ion concentration was 25 mg/l Cr(VI) and incubation period was 48h].

DISCUSSION

Chromite mine overburdens being rich in heavy metals like nickel, chromium, iron and cobalt represent an ideal environment for natural enrichment of heavy metal resistant microorganisms. Several attempts have been

made to isolate chromium resistant bacteria and fungi from such environments (Acharya et al., 1998; Ahmed et al., 2005; Sen and Ghosh, 2007; Dey and Paul, 2013). Recent reports (Ahmed et al., 2005), also suggest that continuous exposure of soil fungi against heavy metals may influence adaptation of fungi to heavy metal concentration.

Chromium resistant fungi isolated from the overburdens of chromite mining sites of Orissa, India, were not exception and showed a high degree of adaptation and tolerance to Cr(VI) and appeared to be potential candidates for Cr-bioremediation (Figure 1). Taxonomically, majority of these isolates belonged to *Aspergillus* and *Penicillium*, however, *Fusarium*, *Mortiriella* and *Trichoderma* were not uncommon.

Considering the comparative inefficiency of live biomass in metal uptake as compared to non-living biomass due to the metabolic interference in a living cell, attempts have been made to utililize the dried mycellial mass of these Cr-tolerant fungal isolates for sorption of Cr(VI). *A. niger* NUA101, the most efficient strain derived from overburden (Table 1), attained an equilibrium of Cr biosorption in 48 h (Figure 2) and corroborates the findings of Mala et al. (2006). However, reports of efficient metal accumulation by live fungal cultures are not uncommon (Srivastava and Thakur, 2006; Congeevaram et al., 2007).

The increase in Cr(VI) sorption by dried biomass of *A. niger* NUA101 with an increase of the initial metal ion concentration (Figure 3) indicated the better efficiency of the organism to sequester more Cr(VI) similar to *Aspergillus* sp. and *Rhizopus* sp.(Ahmed et al., 2005), where Cr biosorption increased with an increase in metal concentration from 2 to 6 mM.

Various adsorption isotherm equations (Langmuir and Freundlich isotherm models) have been used to study the nature of adsorption, with the basic purpose of optimization of the design of adsorption-units for removal of pollutants from the waste waters, (Subramanyam and Das, 2009). The linearised Langmuir and Freundlich adsorption isotherms (Figure 4A, 4B), indicate that adsorption of Cr(VI) on to the dried biomass of *A . niger* NUA101 is favorable, and linear coefficients (R^2) showed a fit between the experimental and the calculated values.

However, the inverse relationship of biomass concentration and Cr(VI) sorbed by *A. niger* NUA101 (Figure 5) supported the observation of Pal et al, (2006) who also reported an inverse relation between concentration of *Morteriella* sp. 403 isolated from the serpentines of Andaman with the rate of Co(II) sorption in solution. In contrast, Prasenjit and Sumathi (2005) and several others (Srivastava and Thakur, 2006; Deepa et al., 2006) concluded that increase in the metal binding sites with increase in biomass concentration is the possible reason for the increment in the rate of biosorption.

Temperature is known to affect the stability of the cell wall, its configuration and can cause ionization of chemical moieties, affecting its metal binding capacity (Gulay et al., 2003). Similarly, biosorption of heavy metals by microbial biomass is strongly pH sensitive and increases as solution pH increases (Yin et al., 1999). Poor sorption at low pH range could be due to the competition of the metal cations with H^+ ions for metal binding sites on the biosorbent surface, while, the increase in pH favors sorption because of the elevated levels of negatively charged groups. At a low pH, some of the functional groups will be positively charged and may not interact with metal ions (Congeevaram et al., 2007). Hasan et al. (2000) reported maximum Ni(II) removal at a pH range of 4.5 to 5.5. The temperature (Figure 6A) and pH optima (Figure 6B) as observed in the present study for Cr(VI) sorption with *A. niger* NUA101 were similar to the observations of Sar et al. (1999) and Deepa et al. (2006).

Presence of additional cations in the solution often affects the rate of metal biosorption. Kovacevic et al, (2000) reported that, kinetics of biosorption appears to be faster in the single-component systems in comparison to the multi-component one. Biosorption of hexavalent chromium by dried biomass of *A. niger* NUA101 changes with the presence of additional cations in the bisorption solution (Figure 7). The reduced uptake of Cr(VI) by the fungal biomass in presence of Ni(II) and Co(II), might be due to the competition of metal cations for complexation with active binding sites of the fungal cell wall. Hussein et al. (2004) reported that the rate of biosorption of Cu(II) is strongly affected by the presence of Cr(VI). This is due to modulation of the outer surface of the fungi by $K_2Cr_2O_7$ facilitating the binding of Cu(II). On the contrary, this non-inhibitory effect of Zn (II) and Fe (III) on Cr(VI) biosorption can be attributed to the difference in ionic radii of the metal with the metal binding sites (Sag and Kutsal, 1996).

Pre-treatment of the biomass often influence the rate of metal biosorption due to alteration of its physico-chemical properties (Ceribasi and Yetis, 2001). According to the present findings (Figure 8), autoclaving and acid pre-treatment have reduced the rates of biosorption similar to those reported by Huang and Chiu (1994) and Kapoor and Viraraghavan (1998). Such reduction in the rate of biosorption could be due to the disorganization of the active metal binding sites on the fungal biomass, whereas, alkali and detergent treatment is said to remove surface impurities exposing more of the metal binding sites and hence enhancing biosorption of metals (Yan and Viraraghavan, 2000).

Conclusion

The chromite mine overburden of Sukinda and Baula-Nuasahi region of Orissa, India, loaded with considerable amount of toxic chromium has resulted in the isolation of chromium resistant *A. niger* NUA101 capable of accumulating significant amount of Cr(VI) in the biomass

through the process of optimization of various process parameters. On the basis of the results obtained, it can be concluded that microbes inherent to the mining environment could be employed as heavy metal decontaminants; however, further in-depth studies are required to optimize the economical requirement.

Conflict of interests

The authors did not declare any conflict of interest.

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