

Biosorption of hexavalent chromium by *Aspergillus fumigatus* S101 isolated from a coal mining environment

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Abstract

In this study attempts were made to isolate a Cr-binding fungal population from coal mine soils of Jharkhand, India. A total of 15 fungal isolates belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium* were obtained from the soils. Among them, *Aspergillus fumigatus* S101 was found to tolerate up to 5 mM Cr(VI) and showed maximum metal uptake capacity of 1.868 mM g⁻¹ biomass in presence of 0.5 mM Cr(VI) within 48 h. The sorption capacity was standardized following Langmuir and Freundlich absorption isotherm models that followed pseudo-second-order kinetics. The biosorption ability of *A. fumigatus* S101 was maximum at pH 5 and at 30 °C. There was a decrease in metal uptake capacity with increase in biomass, but with the increase in Cr(VI) concentration metal uptake capacity increased. The sorption capacity increased in presence of metal ions such as Fe(III) and Cu(II) showing complete removal of 0.5 mM Cr(VI) within 36 h. Treatment of biomass with Tween 80 resulted in maximum sorption accounting for 100% removal of 0.5 mM Cr(VI) in 24 h. The results suggested that the mycelial mass of *A. fumigatus* (S101) is capable of efficient removal of Cr(VI) from aqueous solution.

Key words: absorption isotherm models, *Aspergillus fumigatus*, coal mine soils, Cr(VI) biosorption.

Abbreviations: Cr(VI), hexavalent chromium; Cr(III), trivalent chromium; EDX, energy dispersive X ray analysis; FTIR, Fourier Transform Infrared; MIC, minimal inhibitory concentration; PDA, potato dextrose agar; SEM, scanning electron microscopy.

Introduction

The mining industry is mostly associated with high degree of overexploitation of natural resources for extraction of minerals, which leads to immense environmental pollution and major destruction of habitats. Such overexploitation and physical weathering often results in the release of different types of heavy metals in the environment (Mileusnic et al. 2014).

Waste water discharged from coal mines is usually reported to contain several heavy metals including Cr (Masto et al. 2015), which mainly exists in two stable valance states, the trivalent and the hexavalent. The main difference between these two valance states is that Cr(III) is less bioavailable in the natural environment and therefore less toxic (Rai et al. 1987), whereas Cr(VI) is highly soluble, can permeate cell membrane and is toxic, carcinogenic and mutagenic (Bagchi et al. 2002). Cr(VI) has also been reported to cause oxidative stress in cells by generating reactive oxygen species and subsequently leading to DNA damage and altered gene expression. Therefore, removal of Cr(VI) is essential for mitigation of such contaminated mining areas.

Different physicochemical methods have been applied

for treatment of metal contaminated wastes, but most of these methods are not environment friendly and removal of toxic metals by biological methods such as biosorption, bioreduction and biotransformation has been long preferred as an environment friendly alternative. Several microorganisms, both in dead and living conditions, have been reported to have metal binding and removal ability which can be effectively utilized for mitigation of metal contaminated wastes (Dey, Paul 2010; Jia et al. 2014; Mala et al. 2006). Isolates like *Pseudomonas aeruginosa* (Chatterjee et al. 2011), *Aspergillus niger* and *Micrococcus* sp. (Congeevaram et al. 2007) obtained from tannery effluent and electroplating industry have been previously reported to be effective in biosorption of Cr(VI). A high proportion of cell wall material in fungal biomass has been reported to facilitate the biosorption procedure in both batch as well as continuous cultures. Different species of *Aspergillus* have been reported to be very efficient in biosorption and bioaccumulation of Cr(VI) by different groups of authors (Srivastava, Thakur 2006; Ghosh et al. 2015).

In the present study, soil samples were collected from both abandoned and active coal mines of Jharkhand, India, an area that is rich in different mineral deposits. Mining in this area started as early as 1774 and recent studies

show that continuous mining has led to destruction of the ecological balance and have generated huge amount metal containing mine waste (Manna, Maiti 2017), which has direct impact on the microflora of the region. Previous studies have shown the presence of Cr(VI) in the coal mining areas in the range of 1.09 to 12.1 $\mu\text{g L}^{-1}$ (Mahato et al. 2014), which in some cases exceeds the maximum allowable concentration for Cr (Das, Chakrapani 2011), showing a medium level of heavy metal pollution index. In this study Cr(VI) resistant fungal strains were isolated from the coal mine soils and identified. The ability of these fungal strain in Cr(VI) biosorption ability were quantified in details with the intention of potential application of fungal biomass for removal of Cr(VI) from contaminated water.

Materials and methods

Collection of samples

A total of eight mine soil samples were collected, including two each from Giddi, Religara, Balkundra and Sangam open cast mines of North Karanpura coal fields (23.61 to 23.71° N, 85.27 to 85.46° E) of Jharkhand, India during January in 2017. The open cast mines of the Sangam area was abandoned in 1990, while the others were operating mines. Soils were collected from the subsurface zone in ziplock pouches and stored at 4 °C for further microbiological analysis.

Isolation of fungal strains

Serially diluted soil samples were plated on potato dextrose agar (PDA) plates, amended with streptomycin (Himedia) and incubated for 4 to 6 days at 30 ± 2 °C. Morphologically distinct fungal strains appeared on the plate and were isolated in pure form. The pure cultures were maintained on slopes of PDA at 4 °C and subcultured at regular intervals.

The micromorphological characteristics of the fungal isolates were studied in detail to confirm their generic identity following the Manual of Soil Fungi by Gilman (1957) and Manual of Penicillia (Raper, Thom 1984).

Evaluation of Cr(VI) resistance

The chromium tolerance of these fungal isolates was evaluated following the agar dilution method (Cervantes, Ohtake 1988) in which they were allowed to grow in PDA plates amended with 1.0 to 5.0 mM Cr(VI) at 30 ± 2 °C and the minimum concentration of metal that inhibited growth of the fungal isolate was considered as the minimal inhibitory concentration (MIC) for that particular isolate.

Preparation of fungal biomass

To produce the biomass necessary for biosorption experiment, potato dextrose broth medium (50 mL per 250 mL Erlenmeyer flask) was inoculated with a homogeneous spore suspension prepared from 4-day-old slant cultures of

selected isolate using sterile Tween 80 (0.1% w/v) solution. The biomass was harvested by centrifugation at 6000 \times g for 10 min using a Remi R24 centrifuge and washed with distilled water. This was followed by drying of biomass at 80 °C for 96 h. The biomass was then powdered in a mortar with pestle, sieved through 0.1 mm mesh and stored in desiccators at room temperature for future use.

Biosorption studies

Cr(VI) biosorption was studied using an initial Cr(VI) concentration (as K_2CrO_4) of 0.5 mM in double distilled water (25 mL) containing a biomass level of 2%. The flasks were incubated at 30 °C for 48 h in a rotary shaker (120 rpm) and samples (1 mL) were withdrawn at regular intervals, centrifuged (10 000 \times g for 10 min) and filtered through Whatman No. 42 filter paper. The residual Cr(VI) were estimated by measuring the decrease in hexavalent chromium in the supernatant following the standard 1, 5-diphenylcarbazide method (Park et al. 2000). The Cr(VI) biosorption was represented as Cr(VI) biosorption in mM per g of biomass. All of the experiments were done in triplicate.

Estimation of Cr(VI) biosorption ability

The total Cr(VI) biosorption capacity, designated as q_t , was estimated following the standard mass balance equation

$$q_t = (C_o - C_t) V / W,$$

where C_o (mM) and C_t (mM) designates the initial and the residual Cr(VI) concentration at incubation times $t_o = 0$ h and $t = t$ h, respectively, V designates the solution volume in L and W represents the biomass in g and the biosorption capacity was expressed as Cr(VI) biosorption in mM per g biomass.

Scanning electron microscopic studies

The surface morphology of the biosorbent was studied by scanning electron microscopy (SEM, Zeiss EVO 18). For SEM studies, *A. fumigatus* S101 biomass samples before and after biosorption were dehydrated with 50, 70, 90 and 100% ethanol, mounted on an aluminium stab and coated with a 10-nm thick platinum coating in a Quorum Q150 TES sputter coater for 10 min under vacuum.

Fourier Transform Infrared studies

The chemical nature of the biosorbent surface, before and after adsorption of Cr(VI) was examined by a Fourier Transform Infrared Spectrometer (Perkin-Elmer). Prior to FTIR analysis, KBr pellets were prepared with the biomass and analyzed for any changes of the chemical nature of the biosorption surface.

Kinetic studies

To determine the mechanism of adsorption, the adsorption rates were fitted into pseudo first order and pseudo second order kinetic models as proposed by Lagergren (1898) and

Ho and McKay (1999) respectively.

The pseudo-first-order kinetic model for adsorption of solute from a liquid solution is described by the following equation:

$$\log (q_{eq} - q_t) = \log q_{eq} - k_1 (t / 2.303),$$

where q_t is the amount of Cr(VI) adsorbed at time t [mM Cr(VI) per g biomass]; q_{eq} is the amount of Cr(VI) adsorbed at equilibrium [mM Cr(VI) per g biomass]; and k_1 is the equilibrium rate constant for pseudo-first-order reaction (min^{-1}); and t = time (min).

The adsorption kinetics may also be described by the pseudo-second-order kinetic model expressed by the equation

$$t / q_t = 1 / k_2 (q_{eq})^2 + 1 / q_{eq},$$

where the intercepts and slopes of t / q_t versus t were used to determine the pseudo-second order rate constant k_2 and equilibrium adsorption density q_{eq} , respectively.

Statistical analysis

All experiments were performed in triplicate and results represent mean \pm standard error.

Results

A total of 15 fungal isolates were obtained from the eight coal mine soil samples on PDA plates supplemented with streptomycin. However, fungal isolate was not found in sample S3, which was collected from the active opencast mining site of Giddi colliery. Six different fungal isolates were obtained from sample S1 collected from Religara open cast mines. All of the 15 fungal isolates were identified (Table 1) and screened for their tolerance to Cr(VI). A total of eight fungal isolates were capable of growing even in the presence of 5.0 mM Cr(VI) and were selected as potent Cr(VI) resistant strains (Table 1). The fungal isolates were identified following the Manual of Soil Fungi by Gilman (1957) and Manual of Penicillia (Raper, Thom 1984), and was found to belong mostly to the genera *Aspergillus*, *Fusarium* and *Penicillium* (Table 1). The species of

Table 1. Identification and minimum inhibitory concentration of the Cr(VI) resistant fungal isolates obtained from coal mine soils of Jharkhand, India. *Identification were done according to the Manual of Soil Fungi by Gilman (1957) and Manual of Penicillia (Raper, Thom 1984). **MIC (minimum inhibitory concentration) was determined following agar dilution method using Cr(VI) concentration of 1 to 5 mM

Sample No.	Isolate No.	Identified isolates*	MIC (mM)**
S1	S101	<i>Aspergillus fumigatus</i>	5
	S102	<i>Aspergillus niger</i>	3
	S103	<i>Aspergillus niger</i>	–
	S104	<i>Aspergillus niger</i>	–
	S105	<i>Fusarium oxysporum</i>	5
S2	S201	<i>Aspergillus terreus</i>	5
	S201	<i>Aspergillus terreus</i>	5
S4	S401	<i>Penicillium</i> sp.	5
	S402	<i>Penicillium</i> sp.	–
S5	S501	<i>Aspergillus candidus</i>	5
	S502	<i>Aspergillus koningi</i>	–
S6	S601	<i>Penicillium</i> sp.	5
S7	S701	<i>Penicillium</i> sp.	5
S8	S801	<i>Penicillium</i> sp.	–

Aspergillus were *Aspergillus fumigatus* (S101), *Aspergillus niger* (S102, S103 and S104), *Aspergillus terreus* (S 201), *Aspergillus candidus* (S 501) and *Aspergillus koningi* (S502). Isolates S106, S401, S402, S601, S701 and S801 belonged to genera *Penicillium*.

All these eight fungal isolates were further selected and screened for their Cr(VI) biosorption ability in aqueous solution using dried fungal biomass. The results clearly indicated that the Cr(VI) binding capacity of the fungal biomass varied to different degrees (Table 2). All isolates showed high metal loading potential ranging from 1.524 ± 0.36 to 1.868 ± 0.91 mM Cr(VI) per g of biomass. However, after incubating for 48 h, maximum Cr(VI) biosorption was achieved with *A. fumigatus* S101 biomass, which was able

Table 2. Screening of Cr(VI)-resistant fungi for biosorption of Cr(VI). Initial Cr(VI) concentration (as K_2CrO_4) of 0.5 mM and a biomass level of 2%, incubation: 30 °C at 120 rpm. All experiments were performed in triplicate and results represent mean \pm standard error

Fungal isolate	Cr(VI) biosorption [mM Cr(VI) per g biomass]			
	Time of incubation (h)			
	12	24	36	48
<i>Aspergillus fumigatus</i> S101	1.68 \pm 0.10	1.74 \pm 0.30	1.84 \pm 0.96	1.87 \pm 0.91
<i>Aspergillus niger</i> S102	1.66 \pm 0.12	1.70 \pm 0.28	1.74 \pm 0.56	1.78 \pm 0.13
<i>Fusarium oxysporum</i> S105	1.39 \pm 0.07	1.48 \pm 0.58	1.54 \pm 0.24	1.52 \pm 0.36
<i>Aspergillus terreus</i> S201	1.68 \pm 0.10	1.75 \pm 0.62	1.76 \pm 0.21	1.81 \pm 0.11
<i>Penicillium</i> sp. S401	1.64 \pm 0.12	1.66 \pm 0.11	1.72 \pm 0.34	1.71 \pm 0.32
<i>Aspergillus</i> sp. S404	1.64 \pm 0.04	1.68 \pm 0.56	1.72 \pm 0.16	1.73 \pm 0.19
<i>Aspergillus candidus</i> S501	1.42 \pm 0.07	1.50 \pm 0.92	1.53 \pm 0.42	1.57 \pm 0.31
<i>Penicillium</i> sp. S701	1.48 \pm 0.05	1.60 \pm 0.56	1.75 \pm 0.52	1.76 \pm 0.41

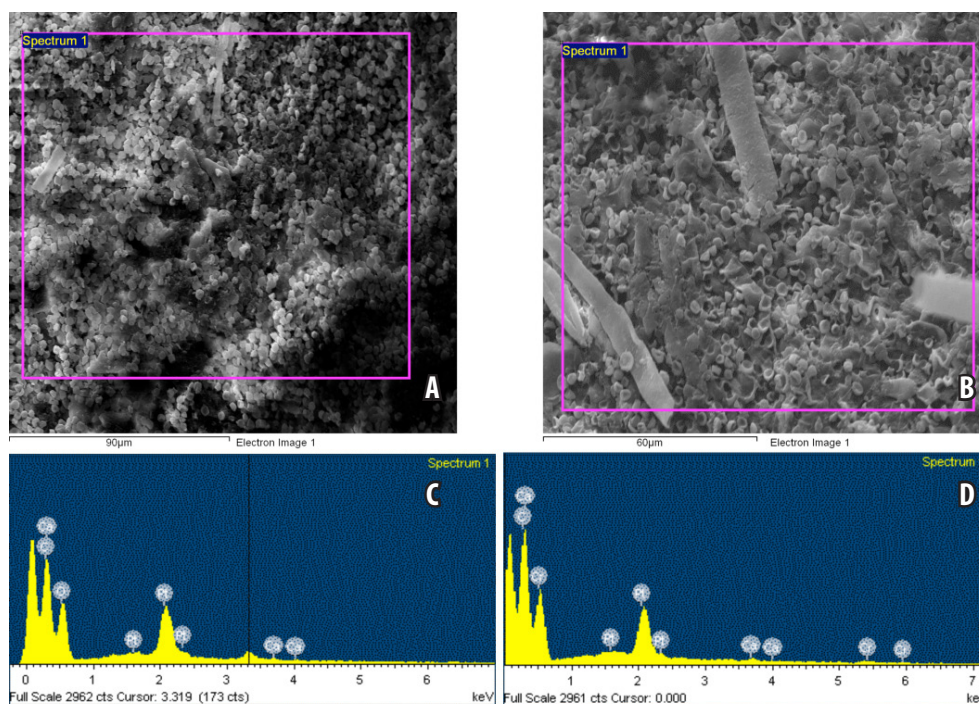


Fig. 1. Scanning electron microscopic images of biomass of *Aspergillus fumigatus* S101 before (A) and after (B) Cr(VI) biosorption. EDX analysis of the same samples before (C) and after Cr(VI) biosorption (D).

to remove nearly 93% of initial 0.5 mM Cr(VI), showing a metal loading capacity of 1.868 ± 0.91 mM Cr(VI) per g of biomass. *A. fumigatus* S101 was, therefore, selected as the best biosorbent and its optimal conditions for Cr(VI) biosorption were evaluated.

The surface morphology of dried and powdered fungal biomass was studied under scanning electron microscopic studies both before and after Cr(VI) biosorption to observe changes. SEM images showed a heterogenous nature of the mycelia (Fig. 1A, B), which can serve as an active site for metal binding. Significant morphological changes were not visible in the SEM image after treatment with Cr(VI). The fungal hyphae appeared to be cylindrical, septate, and branched in nature. The EDX analysis of the biomass after treatment with Cr(VI) confirmed the presence of chromium within the cell mass (Fig. 1 D) suggesting possible attachment of metal on the active sites present in the biomass.

FTIR spectra analysis of the dried fungal biomass before and after Cr(VI) biosorption was done to obtain information about the nature of surface functional groups. A variety of stretching frequency was visible in the spectrum indicating its complex nature (Fig. 2A, B) and distinct change in the spectra after Cr(VI) biosorption was noticed mainly in the range of 1642 to 1038 cm^{-1} . The bands formed at 3388, 1642, 1399 cm^{-1} for unloaded biomass (Fig. 2A) shifted to 3332, 2851 and 1644 cm^{-1} , however, bands at 618 cm^{-1} remained the same.

As the initial Cr(VI) concentration in the metal solution increased from 0.25 to 4.0 mM there was a sharp increase

in the metal loading capacity by *A. fumigatus* S101 biomass. The metal loading capacity of the biomass increased nearly 10 times from 0.902 to 9.28 mM Cr(VI) per g biomass (Fig. 3). However, there was no noticeable increase in metal loading capacity when the Cr(VI) concentration was increased to 8 mM, as the maximum metal loading capacity was found to be around 9.3 mM Cr(VI) per g biomass.

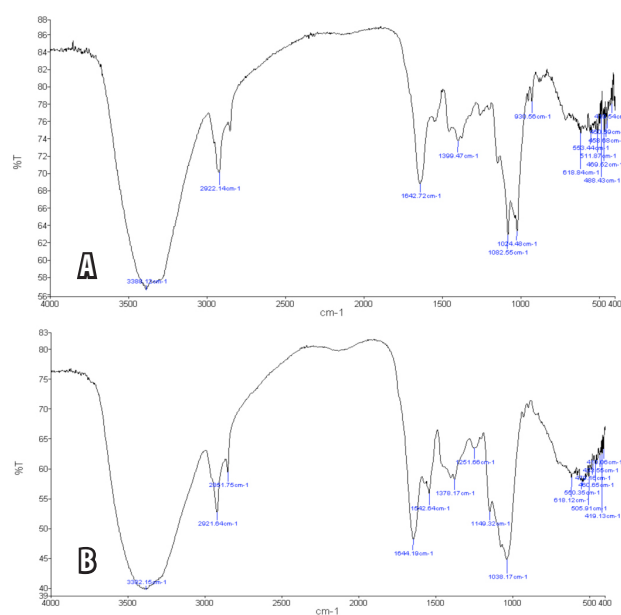


Fig. 2. FTIR analysis of the biomass of *Aspergillus fumigatus* S101 before (A) and after (B) Cr(VI) biosorption.

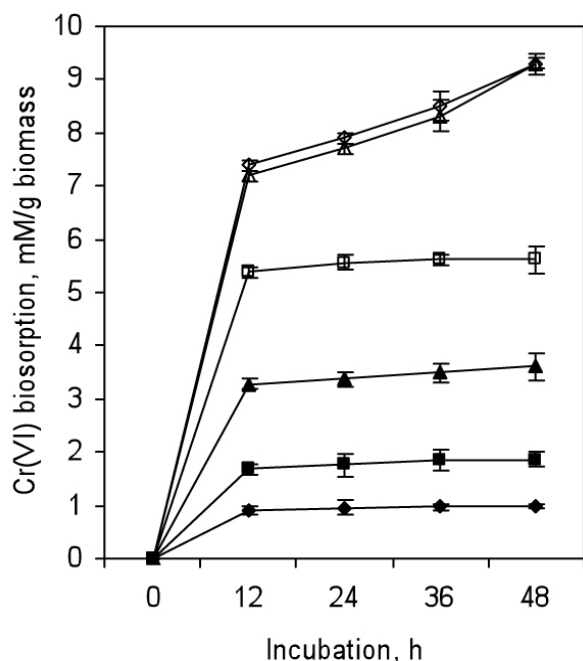


Fig. 3. Effect of sorbent concentration on Cr(VI) biosorption by biomass of *Aspergillus fumigatus* S101 [-♦- 0.25, -■- 0.5, -▲- 1.0, -□- 2.0, -△- 4.0 and -◇- 8.0 mM Cr(VI)]. Biosorption studies were carried out in batch mode with 2% biomass, incubation 30 °C at 120 rpm.

The standard adsorption isotherms such as Langmuir and Freundlich isotherms were used to quantify the biosorption equilibrium following the standard formulae.

Langmuir model: $Q_e = (a) \times (b) \times C_e / 1 + (b) \times C_e$
and the Freundlich model: $Q_e = K (C_e)^{1/n}$,

where Q_e is the amount of metal ion biosorbed at equilibrium per unit weight of biomass, whereas C_e represents the metal ion concentration at equilibrium. Langmuir constants are represented as “a” and “b”, while “K” and “n” represents the Freundlich model constants. The linearised Langmuir and Freundlich adsorption isotherms for Cr(VI) biosorption by *A. fumigatus* S101 are presented in Fig. 4A and B respectively. The adsorption constants along with correlation coefficients were calculated (Table 3) from the isotherms.

The constants for Langmuir model were found to be: (a) and (b) as 6.147 and 0.0811 respectively, while those for Freundlich isotherms were (K) as 2.316 and (n) as 1.4.

The process of biosorption by *A. fumigatus* S101 biomass could be explained with the help of kinetic models. The pseudo-second order kinetic model was tested

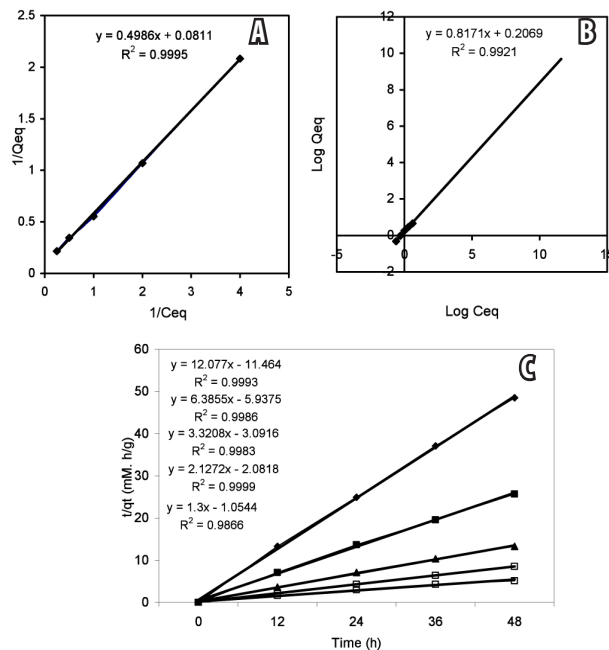


Fig. 4. The linearised Langmuir (A) and Freundlich (B) adsorption isotherms of Cr(VI) by *Aspergillus fumigatus* S101 biomass. (C), pseudo-second order plot (b) for the biosorption of Cr(VI) by *Aspergillus fumigatus* S101 biomass.

with the experimental data to understand the kinetics of Cr(VI) biosorption. The current experiment (Fig. 4C) was in good agreement with the pseudo-second-order kinetic model over all the concentrations studied. The regression coefficient (R^2) was more close to unity, which supported the validity of a pseudo-second order kinetic model for the Cr(VI) biosorption by *A. fumigatus* S101 biomass.

The metal uptake capacity by *A. fumigatus* S101 biomass is strongly influenced by the pH and temperature of the solution. The optimum pH and temperature was found to be 5.0 (Fig. 5A) and 30 °C (Fig. 5B) showing a metal loading capacity of 2.0 and 1.924 mM Cr(VI) per g biomass respectively.

Cr(VI) biosorption capacity of the fungal biomass was influenced by the initial biomass concentration. The maximum Cr(VI) adsorption was achieved when the biomass concentration was kept at the level of 1% where the metal loading capacity was nearly 1.948 mM Cr(VI) per g biomass. However, the metal loading capacity of the dried biomass declined with increase in biomass concentration (Fig. 6).

Table 3. Equilibrium isotherms for Cr(VI) biosorption by *Aspergillus fumigatus* S101 biomass. Q_e , Cr(VI) biosorbed mM Cr(VI) per g biomass; C_e , residual chromium at equilibrium

Model	Equation	Isotherm constant	Corelation coefficient, R^2
Langmuir	$Q_e = 0.4986 C_e / 1 + 0.0811 C_e$	$a = 6.147, b = 0.0811$	0.995
Freundlich	$Q_e = K (C_e)^{1/n}$	$K = 2.316, n = 1.4$	0.992

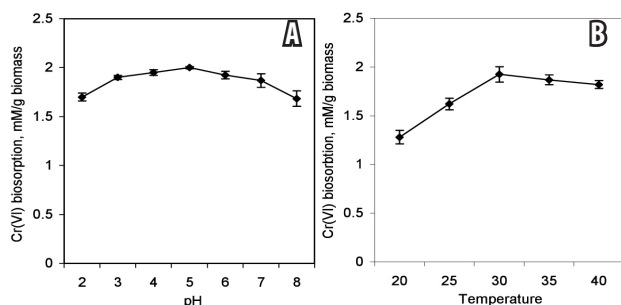


Fig. 5. Effect of pH (A) and temperature (B) on Cr(VI) biosorption by *Aspergillus fumigatus* S101 biomass. Biosorption studies were carried out in batch mode with 2% biomass and 0.5 mM Cr(VI) concentration at 120 rpm for 48 h.

Additional metal cations at equimolar concentration were found to affect the metal loading capacity of the dried biomass of *A. fumigatus* S101. Several metal ions including Ni (II), Zn (II), Co(II), Pb(II), Fe(II) and Cu(II) (as chloride salt) were tested. The results (Table 4) clearly indicated that Ni (II) was most inhibitory, showing Cr(VI) biosorption capacity of 1.280 ± 0.25 mM Cr(VI) per g biomass, accounting for about 68.2% of the control. Presence of Fe (III) ions and Cu(II) was found to promote uptake of Cr(VI) ions by *A. fumigatus* S101 biomass showing a metal loading capacity of nearly 2.0 ± 0.17 mM Cr(VI) per g biomass.

Pretreatment of *A. fumigatus* S101 biomass with various chemical and physical agents have a profound effect on Cr(VI) biosorption and the metal loading capacity. It was found that autoclaving of the sample for 15 min in 103 kPa as well as treatment with 0.5 M sulphuric acid and NaOH was found to reduce the metal loading capacity of the biomass (Table 4). However, pretreatment with 0.5 M Tween 80 was found to promote the Cr(VI) biosorption rate, up to maximum rate of 2 mM Cr(VI) per g biomass after 48 h of incubation.

Table 4. Effect of additional metal cations and pretreatment on Cr(VI) biosorption by biomass of *Aspergillus fumigatus* S101. *Both the initial Cr(VI) and additional metal concentration was kept at 0.5 mM. Incubation period 48 h. Results represent mean of triplicate experiments \pm standard error

Treatment	Cr(VI) biosorption [mM Cr(VI) per g biomass]	Biosorption (% of control)
Control [Cr(VI)]	1.87 ± 0.91	100.00
Cr(VI) + Ni(II)	1.28 ± 0.25	68.20
Cr(VI) + Zn(II)	1.62 ± 0.34	86.18
Cr(VI) + Pb(II)	1.37 ± 0.91	73.09
Cr(VI) + Fe(III)	2.00 ± 0.17	106.40
Cr(VI) + Cu(II)	2.00 ± 0.10	106.40
Autoclaved	1.56 ± 0.24	83.51
0.5 M Tween 80	2.00 ± 0.10	110.20
0.5 M H ₂ SO ₄	1.28 ± 0.04	75.09
0.5 M NaOH	1.20 ± 0.21	64.23

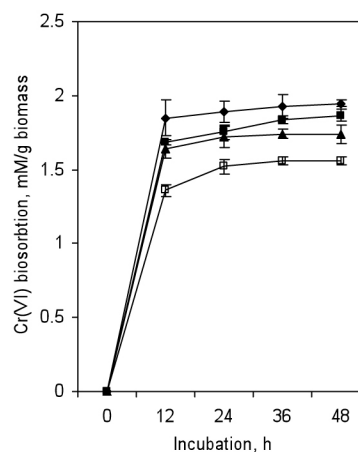


Fig. 6. Effect of initial biomass concentration on Cr(VI) biosorption by biomass of *Aspergillus fumigatus* S101. [-♦- 1, -■- 2, -▲- 3, -□- 4% biomass]. Biosorption studies were carried out in batch mode after 48 h incubation using an initial Cr(VI) concentration of 0.5 mM at 30 °C.

Discussion

The soil samples in and around coal mining areas have been reported to contain several heavy metals such as chromium, lead, zinc and nickel (Khan et al. 2017; Manna, Maiti 2017). The indigenous microbiota obtained from the soil of such mining areas was found to tolerate different types of heavy metals. Similar results were also made by Upadhyay et al. (2017), where they reported presence of metal tolerant microbes from degraded soils of coal mining areas of Uttar Pradesh.

A total of 15 fungal isolates were obtained from these soil samples and majority of these isolates taxonomically belonged to genus *Aspergillus*, *Penicillium* and *Fusarium* sp. (Table 1). Similar observations were also made by Ahmad et al. (2005) and Zafar et al. (2007) who reported the presence of *Aspergillus* sp. as the most common genus in heavy metal contaminated soils.

Among the 15 isolates, eight were found to be tolerant to 5 mM Cr(VI) (Table 1), which is common among the isolates obtained from metal contaminated mining areas. Similar metal tolerance was also reported by Ahmad et al. 2005 in *Aspergillus* sp. and it was suggested that continuous exposure to heavy metals can lead to the development of tolerance in indigenous fungal populations.

The isolates that tolerated up to 5 mM Cr(VI) were further screened for their ability for Cr(VI) biosorption. *A. fumigatus* S101 was found to be a very suitable for biosorption (Table 2) with a metal loading capacity of 1.868 ± 0.91 mM Cr(VI) per g biomass when the initial Cr(VI) was 0.5 mM. This isolate was thus considered as the most efficient strain and selected for further optimization. Similar efficiency of *Aspergillus* sp. in Cr(VI) biosorption was also reported by Ghosh et al. (2015) where the biosorption was reported to be nearly 17.58 mg Cr(VI) per g biomass. Sivakumar (2016) reported biosorption of Cr(VI) up to 18.1 mg L⁻¹ from tannery effluents by *Aspergillus* species such as *A. niger*, followed by *Aspergillus flavus*, *A. fumigatus*, *Aspergillus nidulans*, *Aspergillus heteromorphus*, *Aspergillus foetidus* and *Aspergillus viridinutans*. Similarly, Singh et al. (2016) reported up to 16.1 mg g⁻¹ Cr(VI) biosorption by *A. flavus* and is suitable for use in pilot plants for removal of Cr(VI).

Scanning electron microscope analysis and EDX study of the dried fungal biomass both before and after Cr(VI) biosorption were done and EDX analysis showed the presence of Cr bound to the fungal biomass (Fig. 2B). However, no distinct change in biomass morphology was noticed. The biosorbed chromium was assumed to be trivalent in nature, as Cr(VI) is reduced to insoluble Cr(III) which can freely bind to these sites. After binding Cr(III) is also reported to help in further heterogeneous nucleation and crystal growth (Park et al. 2005).

FTIR spectral analysis of the biomass also provided vital information regarding the functional group responsible for Cr(VI) biosorption. It has been usually found that anionic ligands such as phosphoryl, carbonyl, sulfhydryl and hydroxyl groups contribute greatly to the biosorption processes. Fungi are composed of polysaccharides such as β -glucan, chitin and chitosan, glycoproteins, lipids, D-galactosamine polymers and polyuronides, which act as sites of metal binding (Vimala, Das 2011). In the present case there was a change in the spectra after Cr(VI) biosorption by the fungal biomass mainly in the range of 1642 to 1038 cm⁻¹ (Fig. 3A, B), which indicates the presence of -OH and -N-H ionizing groups as the main functional groups playing a vital role in biosorption. Bai and Abraham (2001) and Khambhaty et al. (2009) suggested that Cr(VI) anions interacted strongly with positively charged amines of the cell wall.

Increased initial metal concentration was found to positively influence the metal loading capacity of the dried biomass (Fig. 4), which indicates the efficiency of

the biomass to load higher concentration of metals due to availability of more metal binding sites. Such increase in biosorption potential can also be associated with increase in propelling force of higher Cr(VI) ion concentration, which was more efficient to overcome the mass transfer resistance of metal ions and subsequently resulting in higher collision between Cr(VI) ions and biosorbents (Tewari et al. 2005; Ahmad et al. 2005; Aravindhana et al. 2012).

Biosorption isotherms equations are used to study the nature of biosorption and to analyze the interrelationship between the mass of the adsorbed component and the concentration of a metal in wastewater (Mandal, Roy 2016). The present study was in good agreement with both Langmuir and Freundlich isotherms, as evident by the high R² value (Fig. 5A, B).

Cr(VI) biosorption by *A. fumigatus* S101 biomass (Fig. 5C) was in good agreement with the pseudo-second-order kinetic model as the regression coefficient (R²) was more close to unity for all the different concentration used. Similar results were also reported by Bose et al. (2011) and Mondal et al. (2017) using *Jatropha* seed press cake and *A. niger*, respectively.

Biosorption of Cr(VI) was largely dependent on the pH of the solution and in the present case was found to increase at lower pH. Maximum biosorption was observed at pH 5 (Fig. 6 A) and Cr(VI) biosorption efficiency gradually reduced at both lower and higher pH. Lower or acidic pH has been reported to enhance the competition between metal ions and H⁺ for metal binding sites of the fungal cell wall, thereby decreasing metal binding efficiency. A similar increase in removal of Ni(II) at pH 4.5 to 5.5 was reported by Hasan et al. (2000). Optimum temperature was found to be 30 °C, where metal loading capacity was found to be nearly 1.924 mM Cr(VI) per g biomass showing the interaction to be endothermic in nature. In the present study, with increase in temperature Cr(VI) biosorption ability was reduced. A similar phenomenon was also reported by Pal et al. (2006) and Choudhury et al. (2012), which is characteristic of a chemical reaction or bond being involved in the adsorption process (Mohapatra et al. 2010).

Initial biomass concentration was also found to effect the Cr(VI) removing ability of the isolate. In the present study, with an increase in biomass concentration from 1 to 3% the biosorption efficiency decreased (Fig. 7). Ghosh et al. (2015) reported a similar phenomenon with *A. niger*, where maximum biosorption occurred at 1 g L⁻¹ and with increase in biomass the biosorption ability decreased. Similar observations were noted for uptake of Cr(VI) by *Aspergillus* (Al-Ashesh, Duvnjak 1995).

Addition of different metal ions along with Cr(VI) was found to have a profound effect on the biosorption potential. The reduced uptake of Cr(VI) in presence of metals such as Ni(II), Pb(II) and Zn(II) (Table 4) was evident in the present study, which may be explained by competition between the metal ions for active sites of the cell wall. The

biosorption was not inhibited in presence of Fe(III) and Cu(II), which may be attributed to the difference in ionic radii of the metal and metal binding site (Sag, Kutsal 1996).

Pretreatment with chemical as well as physical method also affected the biosorption potential. Autoclaving as well as acid and alkali treatment of the biomass reduced the biosorption ability of *Aspergillus* biomass, which was due to disorganization of metal binding sites (Ghosh et al. 2015; Kapoor et al. 1999).

In conclusion, in the present study *A. fumigatus* (S101) was isolated from the coal mine soils of Jharkhand, India. This strain showed tolerance to Cr(VI) and is capable of biosorption of Cr(VI) from aqueous solutions under different conditions. The isolate can be further utilized to remove Cr(VI) from Cr(VI) contaminated waste water. However, further detailed studies are necessary to assess the economic feasibility of the process.

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