HAYATI Journal of Biosciences 24 (2017) 65–71

HOSTED BY

Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original Research Article

Optimization of Chromium Biosorption by Fungal Adsorbent, *Trichoderma* sp. BSCR02 and its Desorption Studies

John Rose Mercy Benila Smily, Pasumalai Arasu Sumithra*

Department of Microbiology, Srimad Andavan Arts and Science College, Tiruchirappalli 620 005, TN, India.

ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form
16 July 2017
Accepted 22 August 2017
Available online 14 September 2017

KEYWORDS:
fungal biosorbent,
heavy metal resistance,
reusability,
18S rRNA sequence analysis,
scanning electron microscope

ABSTRACT

Heavy metal pollution in water because of the discharge of industrial effluent imposes serious environmental concern. Chromium is one of such pollutants which is considered as toxic, non-biodegradable and persistent in nature. In the present study, the fungal strains isolated from the water samples of Manjakkudi lake were screened for their resistance towards the heavy metal, chromium. The *Trichoderma* sp. BSCR02 showing resistance towards increased chromium concentration (4 mg/mL) was selected for the biosorption studies. The chromium biosorption ability of the untreated and alkali-treated mycelium of *Trichoderma* sp. BSCR02 was compared and found the alkali treatment as better biosorbent. The process parameters governing chromium biosorption by the dead biomass of *Trichoderma* sp. were optimized and maximum chromium removal was observed at pH 5 with 200 mg/L initial metal concentration at 35°C when supplemented 1.6 mg/mL of biosorbent for the contact time of 120 min. The biosorbent was found to be active for five cycles of biosorption. The results revealed the applicability of the *Trichoderma* sp. BSCR2 for the effective removal of chromium from the contaminated water bodies.

1. Introduction

In the recent decades, the heavy metal pollution has been considered as the serious environmental problem (Sameul *et al.* 2015). Chromium contamination in the aquatic ecosystems in close vicinity to residential areas has become a serious environmental as well as health issue (Netzahuatl-Muñoz *et al.* 2015). Chromium exists in different oxidation states in aqueous environment, including trivalent and tetravalent. The trivalent chromium [Cr(III)] is considered as an essential trace element for the metabolism in mammals, whereas the tetravalent chromium [Cr(VI)] is considered as toxic, teratogenic and carcinogenic in mammals (Puentes-Cárdenas *et al.* 2012). The increased levels of chromium intake may results in the damage of organs including liver and kidney, weakening of immune system, respiratory disorders, etc. (Viamajala *et al.* 2004; Rakhunde *et al.* 2012).

The removal of tetravalent chromium from the environment or their reduction into trivalent chromium is hence essential in the present scenario. There are number of technologies available to remove chromium from aquatic environment, including ion exchange separation, membrane separation, chemical precipitation, filtration, etc. These methods are expensive, labour consuming and also generate secondary wastes which are difficult to manage. Also, these methods were found to be ineffective when heavy metals are present in lower concentrations (Rakhunde *et al.* 2012). The adsorption of heavy metals using suitable adsorbents has gained great attention towards their remediation. At present, activated carbon is served as commercial adsorbent for the adsorption of heavy metals from contaminated aqueous effluents. The commercial activated carbon was found to be less effective towards the adsorption of tetravalent chromium (Khezami and Capart 2005). Hence, there is a need for the cheap adsorbent material with high adsorption efficiency. Different researchers were analyzing the usage of fruit peel wastes and shell wastes as adsorbent materials, but the success rate was found to be moderate (Doğan *et al.* 2009; Aranda-García *et al.* 2014).

In the recent years, a great attention was paid towards the application of biological materials as adsorbent (biosorbent). The biosorbent is found to be readily available, cheap and possesses great adsorption efficiency (Aravindhan *et al.* 2012). The bacteria, live and dead biomass of fungi, compounds extracted from biological materials and modified biomaterials were currently used as biosorbent (Saha and Orvig 2010; Samuel *et al.* 2015). The fungi

* Corresponding author.
E-mail address: sumithrachandramohanmb@gmail.com (P.A. Sumithra).
Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2017.08.005

possess great advantages over other biosorbent materials in terms of their biosorption and bioaccumulation efficiency because of their cell wall material that shows metal binding properties. In the present investigation, the biosorption of tetravalent chromium was performed using the chromium-resistant fungal isolate *Trichoderma* sp. BSCR02 isolated from Manjakkudi lake. The chromium biosorption ability of live biomass of *Trichoderma* sp. BSCR02 and alkali-treated biomass was evaluated. In addition, the operation parameters governing the biosorption including pH 3, temperature, initial metal concentration, biosorbent concentration and contact time were optimized for the enhanced biosorption of chromium.

2. Materials and Methods

2.1. Sampling and physicochemical characterization of sample

Manjakkudi is the small village located in Pudukkottai District, Tamil Nadu, which was selected for the present study. The drinking water resource (Manjakkudi lake) of the Manjakkudi was contaminated because of the discharge of both domestic and industrial wastewater. The water samples of 1-m depth from three different locations were collected from the Manjakkudi lake using sterile containers and immediately brought to laboratory for further processing. The water samples were stored in ice packs and preserved at 4°C during their transport to the laboratory for analysis to ensure no change occurs in the composition of samples till analysis. All the physicochemical analyses were carried out in accordance with international standard methods. The results were expressed as mean results of all the three samples.

2.2. Isolation of chromium-resistant fungi

The chromium-resistant fungal strains were isolated from the water samples using potato dextrose broth (PDB). To isolate chromium-resistant fungi, the water samples were spread over the plate on PDB medium amended with potassium dichromate at the concentration of 100 mg/L. The plates were incubated at 37°C for 48 h for the enumeration of chromium-resistant fungi. After incubation, six morphologically different fungal colonies were isolated and labelled as BSCR01−BSCR06. The isolates were purified and used for heavy metal tolerance investigations.

2.3. Molecular identification of chromium-resistant fungi

The pure cultures of the chromium-resistant fungal isolates were grown in PDB overnight and the isolation of genomic DNA was performed using the cetrimide tetradecyl trimethyl ammonium bromide method described by Hiney *et al.* (1992). From the isolated genomic DNA, the internal transcribed spacer (ITS) regions (18S rRNA) were amplified using the universal primers, forward (ITS1-5′-TCC GTA GGT GAA CCT GCG G-3′) and reverse (ITS4-5′-TCC TCC GCT TAT TGA TAT GC-3′) (Bakri *et al.* 2010). The amplified PCR product was purified using Qiagen gel extraction kit (Qiagen, India) and sequenced as described by Singh *et al.* (2014). The isolated chromium-resistant fungal isolates were ascertained to their taxonomic position by sequence similarity search of the amplified 18S rRNA using BLAST search (Zhang *et al.* 2000). Multiple sequence alignment of the 18S rRNA sequences was performed using Clustal W (European Bioinformatics Institute, UK), and phylogenetic distance tree matrix was generated from the data set (Singh *et al.* 2014).

2.4. Heavy metal tolerance assay

To determine the heavy metal tolerance of the isolates, potato dextrose agar medium was prepared with different concentrations of chromium *via*. potassium dichromate, ranging from 1 to 5 mg/L. The fungal isolates were spot inoculated into each freshly prepared chromium plates and incubated for 24 h. After incubation, the extent of chromium was monitored *via*. the mycelial growth of the fungal isolates. The metal tolerance index (*Ti*) of the fungal isolates was determined using the formula:

$$Ti = \frac{Dt}{Du}$$

where, Dt is the radial extension of treated colony in cm and Du is the radial extension of untreated colony in cm. The isolate BSCR02 which showed increased *Ti* till 6 mg/L was further investigated for biosorption studies.

2.5. Preparation of fungal biosorbents

For the chromium biosorption, the live and alkali-treated biomass of *Trichoderma* sp. BSCR02 were assessed. The fungal isolate was inoculated into two Erlenmeyer flasks containing 100 mL of PDB and incubated at 37°C for 7 days. After incubation, the fungal mat from the first flask was harvested and filtered. The fungal mat was washed with distilled water for five times and the live biomass was stored at −20°C. The fungal mat from the second flask was harvested and washed as earlier. The fungal mat was then added with 0.1 N sodium hydroxide (alkali) for 30 min and the pH was neutralized by washing with distilled water. The alkali-treated biomass was then autoclaved, dried at 60°C and powdered.

2.6. Biosorption studies

The chromium stock solution was prepared by dissolving 1000 mg of potassium dichromate in 1 L of deionized water. From stock, chromium ion solution was prepared in two sets at the concentration of 50 mg/L and the pH was adjusted to 7. About 0.5 g of the live and alkali-treated fungal biomass were added separately to the 100 mL of chromium ion solution and the reaction mixtures were incubated in shaker for 60 min. After incubation, the chromium concentration in the reaction mixture was estimated (Sugasini and Rajagopal 2015).

2.7. Scanning electron microscope analysis

The surface topography of the alkali-treated biomass (biosorbent) before and after the biosorption was characterized using scanning electron microscope (SEM) analysis.

2.8. Estimation of chromium

About 5 mL of the above reaction mixture was centrifuged at 4000 rpm for 15 min and the supernatant was used for the estimation of chromium. To 1 mL of the supernatant, 9 mL of 0.2 M sulphuric acid and 0.2 mL of 0.25% diphenyl carbazide in acetone were added, and the absorbance of the pink colour developed was read at 540 nm using distilled water as blank. The linear regression of the standard graph was used for the estimation of chromium present in the solution. The chromium removal percentage was calculated using the following formula:

$$\text{Chromium removal percentage} = \left(\frac{Ci - Cf}{Ci}\right) \times 100$$

where, Ci is the initial chromium concentration in mg/L and Cf is the final chromium concentration in mg/L.

2.9. Optimization of biosorption studies

Because the biosorption using alkali-treated biomass showed increased biosorption percentage, it was used for the optimization studies. The effect of pH on the chromium biosorption using dead biomass of *Trichoderma* sp. BSCR02 was investigated on a wide pH ranged from 3 to 9. The biosorption was carried out in a wide range of temperature (30−50°C) to determine the role of temperature in

chromium biosorption. The effect of initial metal ion concentration was also altered (25–250 mg/L) and tested for biosorption efficiency. The dose of biosorbent also investigated with varying concentration of dead fungal biomass (0.2–2 mg/mL). The contact time of the biosorbent with the metal ion solution (30–180 h) was also optimized further.

2.10. Desorption studies and reusability of the biosorbent

It is essential to remove the metal ions from the biosorbents after biosorption. Hence, the desorption experiments were carried out with the desorping agent HCl. The dead fungal biomass after biosorption was collected and weighed. The desorbing reagent was prepared at the concentration of 0.5 M and the known weight of adsorbed fungal biomass was added and kept in shaker for 24 h. After incubation, the reaction mixture was centrifuged and the supernatant was subjected to the estimation of chromium (desorbed), and the pellet containing desorbed fungal mass was weighed. The desorbed biosorbent was subjected to fresh biosorption studies and the process was repeated for 10 times and subsequent desorption studies were carried out.

3. Results

The surface water samples of the Manjakkudi lake was collected and the physicochemical parameters were characterized using standard methods. The results of the physicochemical characterization of the water samples were displayed in Table 1. The water sample was little turbid in nature. The temperature of the water was found to be 26°C at the pH of 5.7, which indicated that the water was weakly acidic in nature. The dissolved oxygen of the Manjakkudi lake was found to be 8.7 mg/L. The total solids, total dissolved solids and total suspended solids of the water sample were calculated as 31.67, 30.26 and 1.27 mg/L, respectively. The concentration of heavy metals including cadmium (Cd), mercury (Hg), chromium (Cr) and lead (Pb) present in the water samples were determined further. Among the heavy metals, the concentration of chromium was found to be higher (0.08 mg/L) than that of other heavy metals followed by lead (0.003 mg/L).

The population of chromium-resistant fungi present in the water sample was assessed using chromium agar plates. A total of six fungal isolates which possess the ability to tolerate up to 100 mg/L of chromium were selected and labelled as BSCR01, BSCR02, BSCR03, BSCR04, BSCR05 and BSCR06. The fungal isolates were selected based on their degree of resistance towards the chromium (Figure 1).

The sequence of the 18S rRNA gene was deduced for all the six chromium-resistant fungal isolates. The highly similar sequences of the deduced sequences were aligned using BLAST search. All the 18S rRNA sequences of the fungal isolates were closely related to the sequences of previously reported fungal strains, with an average identity of 99%. Based on the 18S rRNA sequence analysis,

Table 1. Physicochemical characterization of water samples from Manjakkudi lake

Parameter	Range
Temperature (°C)	26 ± 0.55
pH	5.7 ± 0.39
Dissolved oxygen (mg/L)	8.7 + 0.43
Total solids (mg/L)	31.67 ± 2.98
Total dissolved solids (mg/L)	30.26 ± 3.42
Total suspended solids (mg/L)	1.27 ± 0.12
Cadmium (mg/L)	0.001 ± 0.0004
Mercury (mg/L)	0.001 ± 0.0006
Chromium (mg/L)	0.08 ± 0.002
Lead (mg/L)	0.003 ± 0.0008

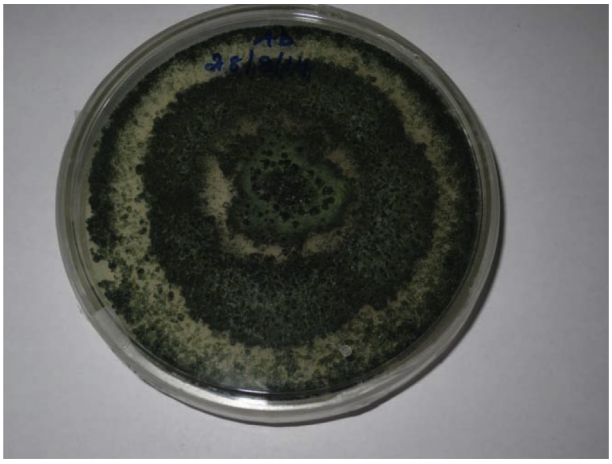

Figure 1. *Trichoderma* sp. BSCR02 grown in PDA plate. PDA = potato dextrose agar.

the isolates were identified as *Aspergillus terreus* (BSCR01), *Trichoderma* sp. (BSCR02), *Penicillium* sp. (BSCR03), *Trichoderma gamsii* (BSCR04), *Trichoderma amazonicum* (BSCR05) and *Penicillium decumbens* (BSCR06). The sequences were submitted to GenBank database and obtained accession numbers (Table 2). The distance tree results revealed the relatedness between the isolated fungal isolates (Figure 2).

The fungal isolates showing tolerance index above 0.6 were found to be resistant towards chromium (Table 3). Among the isolates, the *Trichoderma* sp. BSCR02 was found to show tolerance up to 4 mg/L chromium concentration. Next to *Trichoderma* sp. BSCR02, the isolate *A. terreus* BSCR01 was found to tolerate up to 3 mg/L chromium concentration. The *P. decumbens* BSCR06 was identified as least resistance towards chromium (2 mg/L).

The biosorption capacity of the live and dead biomass of *Trichoderma* sp. BSCR02 was tested against chromium under standard operational conditions. The alkali-treated biomass absorbed maximum amount of chromium when compared with the live biomass (Table 4). The biosorption capacity of the dead biomass (78.4%) was found to be greater than that of the live biomass (65.7%).

The SEM micrographs of the dead fungal biomass before (Figure 3A) and after (Figure 3B) the chromium biosorption were compared. The SEM micrographs of fungal biomass possess a highly porous matrix and a large surface area. There observed a visible difference in the SEM micrographs between the surface topography of treated and untreated biosorbents with few morphological disintegrations.

The effect of pH on chromium biosorption by the dead biomass of *Trichoderma* sp. BSCR02 was studied. Among the pH studied, the maximum removal of chromium (82.3%) was observed at the pH 5 (Figure 4). With the increase in pH beyond 5, no biosorption of chromium by the dead fungal biomass was observed. Next to pH, temperature plays a crucial role in the biosorption of heavy metal, which affects the stability of cell wall composition and

Table 2. List of chromium-resistant fungi isolated with their accession numbers

Isolate	Accession number	Name of the organism
BSCR01	KX036368	*Aspergillus terreus*
BSCR02	KX036369	*Trichoderma* sp.
BSCR03	KX036370	*Penicillium* sp.
BSCR04	KX218455	*Trichoderma gamsii*
BSCR05	KX218456	*Trichoderma amazonicum*
BSCR06	KX218457	*Penicillium decumbens*

Figure 2. Phylogenetic relationship between the isolated chromium-resistant fungi.

Table 3. MICs of isolated fungal strains

Name of the organism	MIC (mg/L)
Aspergillus terreus (BSCR01)	3
Trichoderma sp. (BSCR02)	4
Penicillium sp. (BSCR03)	2
Trichoderma gamsii (BSCR04)	1
Trichoderma amazonicum (BSCR05)	2
Penicillium decumbens (BSCR06)	1

MIC = minimum inhibitory concentration.

Table 4. Biosorption efficiency of live and alkali-treated fungal biomass

Biomass	Chromium initial concentration (mg/L)	Chromium remained (mg/L)	Chromium adsorbed (mg/L)	Chromium biosorption efficiency (%)
Live biomass	50	11.32	38.68	77.36
Dead biomass	50	8.37	41.63	83.26

configuration (Gulay *et al.* 2003). The maximum biosorption of chromium (86.49%) by the dead fungal biomass of *Trichoderma* sp. BSCR02 was observed at 35°C (Figure 5).

The chromium biosorption ability of the dead fungal biomass of *Trichoderma* sp. BSCR02 was analyzed with different concentrations of chromium. The experiments were carried out in optimized pH and temperature. The biosorption efficiency of the fungal biosorbent was gradually increased with the increase in initial metal concentration up to 200 mg/L (Figure 6). A sudden decrease in the chromium biosorption was observed beyond the optimum initial metal concentration. The biosorption efficiency of the fungal biosorbent increases with the increase in the biosorbent concentration. The maximum biosorption (93.47%) was observed with the 1.6 mg/mL concentration of the fungal biosorbent (Figure 7). Generally, lower biosorption was observed with the lower concentrations of biosorbent (Vijayaraghavan *et al.* 2006). The effect of contact time on the chromium biosorption was studied at different incubation periods. The maximum biosorption was observed during 120 min of contact time (96.38%) and no further increase in the biosorption efficiency up to 180 min (Figure 8).

In the present study, 100% of the adsorbed chromium was desorbed with the help of desorbents such as HCl. The 0.5 M concentration of HCl was enough for the complete desorption of chromium from the adsorbents. The biosorption experiment was repeated for evaluating the reusability of biosorbent. There is a decrease in the biosorption efficiency of the biosorbent after five cycles and found to be constant beyond that (Table 5). The results revealed that the dead fungal biomass of *Trichoderma* sp. BSCR02 could be repeatedly used as an adsorbent for the biosorption of chromium up to five cycles without losing its adsorption capacity.

4. Discussion

In the present investigation, Manjakkudi lake was selected because it served as a drinking water resource for the nearby villages and frequently gets contaminated by domestic and industrial effluents. The Manjakkudi village is located in Manamelkudi Tehsil of Pudukkottai District and populated with around 1500 people whose primary occupation is agriculture. The physiochemical characterization of the water samples was performed using standard methods. The pH and temperature of the lake water might favour the growth of microorganisms as most of the freshwater microorganisms were mesophilic and grown in the pH range of 4–9 (Pandit *et al.* 2013). The biological life of the water bodies is greatly depended on the dissolved oxygen and is considered as an

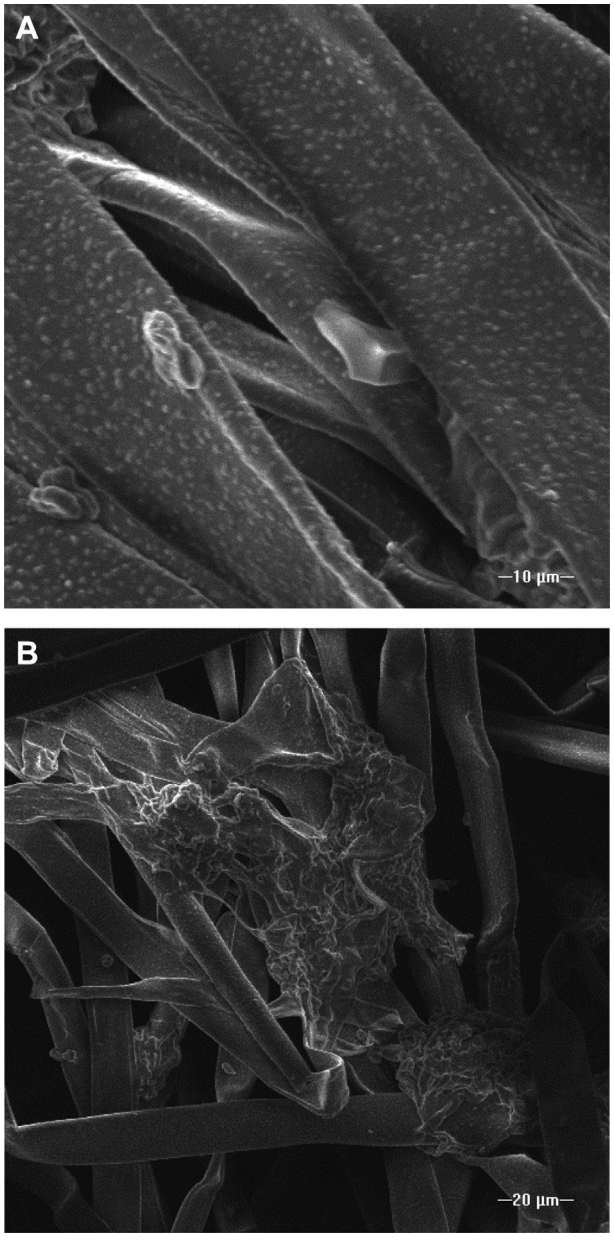

Figure 3. (A) SEM micrograph of dead fungal biosorbent. (B) SEM micrograph of chromium adsorbed to biosorbent. SEM = scanning electron microscope.

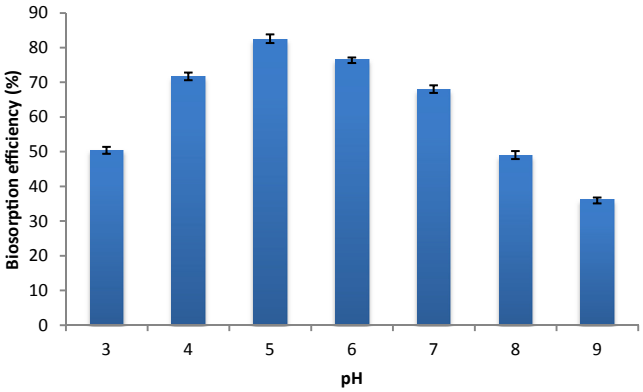

Figure 4. Effect of pH on chromium biosorption by fungal biosorbent.

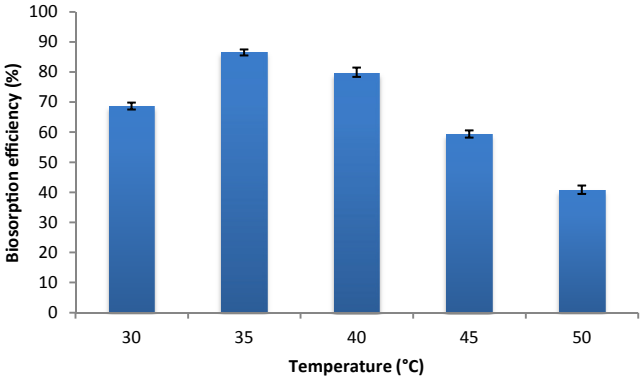

Figure 5. Effect of temperature on chromium biosorption by fungal biosorbent.

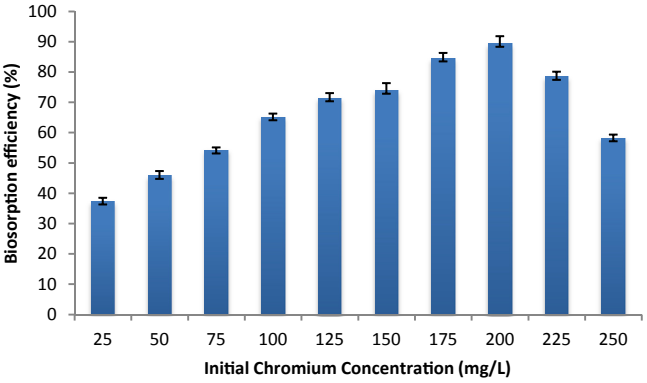

Figure 6. Effect of chromium (potassium dichromate) concentration on chromium biosorption by fungal biosorbent.

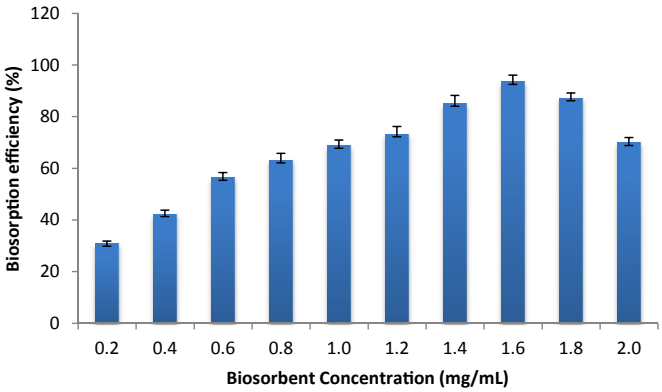

Figure 7. Effect of biosorbent concentration on chromium biosorption by fungal biosorbent.

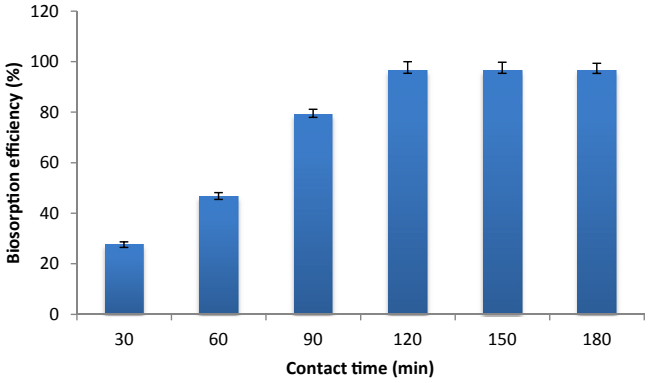

Figure 8. Effect of contact time on chromium biosorption by fungal biosorbent.

Table 5. Reusability studies of fungal biosorbent

Cycle number	Biosorption efficiency (%)	Desorption (%)
1	96.5	94.2
2	95.2	93.7
3	94.9	94.6
4	94.6	93.8
5	94.6	92.5
6	91.5	95.8
7	89.84	94.7
8	81.73	93.0
9	76.35	93.6
10	70.62	92.7

essential factor to determine water quality (Barman *et al.* 2001). The results of similar range were observed by Norzatulakma (2010) and Sujaul *et al.* (2013). The heavy metal contamination in the Manjakkudi lake water was also assessed and found chromium to be predominant. The concentration of mercury and cadmium were too low in the water samples of Manjakkudi lake. The major sources of chromium contamination in the surface water include tanneries, chemical industries, electroplating industries, iron and steel foundries, etc. (Eisler 1986; Stasinakis *et al.* 2003).

Microorganisms including fungi are not only known to possess heavy metal tolerance, but they are also known to have heavy metal binding abilities and also their detoxification (Sardrood *et al.* 2013).

The chromium-resistant fungal isolates were morphologically characterized and identified using 18S rRNA sequence analysis. The molecular identification of fungi using phylogenetically informative target, such as 18S rRNA served as an accurate and definitive fungal identification method (Singh *et al.* 2014). The results revealed that the fungal isolates belonging to the species of *Trichoderma* were found to be predominant among the isolated chromium-resistant fungi. Reports were available evidencing the bioaccumulation ability of *Trichoderma* sp. towards different heavy metals in substantial amounts (Volesky 1990; Sen and Ghosh 2010).

To select the best fungal isolate for the biosorption studies, heavy metal tolerance ability of the isolated fungal isolates was evaluated. In a previous report, *Aspergillus niger* was found to possess heavy metal resistance up to 2 mg/L with *Ti* of 0.75 (Santhi and Guru 2014). The reports also evidenced that the alkali-treated

biomass was found to be efficient than that of the live fungal biomass. The reason behind the decreased biosorption efficiency of the live biomass might be due to their active cellular metabolism which hinders their biosorption efficiency. The dead biomass in the absence of metabolic activities possesses greater metal binding capacity (Gochev Velizar *et al.* 2010). The SEM results of the present investigation support the findings of the earlier researchers (Sethuraman and Balasubramanian 2010). A similar trend of morphological difference was observed during the biosorption of cadmium by the dead biomass of *Aspergillus aculeatus* DBF9 (Pandey and Banerjee 2011).

The pH plays an important role in the biosorption of heavy metals. The lower pH causes the protonation of binding sites in microbial surface, and thereby imparts negative charge to microbial surface, which in turn contributing metal binding (Shankar *et al.* 2007). The lower pH of 5 was found to be optimum for the biosorption of different heavy metals such as lead using *Rhizopus nigricans* (Yan and Viraraghavan 2003), Nickel by Pencillium *chrysogenum* (Tian-Wei *et al.* 2004) and copper by *Micrococcus luteus* (Leung *et al.* 2000). In similar reports, the reduction in metal absorption was observed in alkaline pH (Nasseri *et al.* 2002). Goyal and Banerjee (2003) and Shankar *et al.* (2007) also reported similar trend of biosorption of chromium using *Streptococcus equisimilis* and *Aspergillus* sp., respectively.

The differences in the biosorption with respect to the metal concentration might be due to the loss of equilibrium in the reaction mixture, and thereby affects the electrostatic interactions between the biosorbent and the metals (Iqbal and Edvean 2004). The biosorbent dosage plays a primary role in the heavy metal biosorption. The concentration of fungal biosorbent was also optimized for the better chromium biosorption. The increase in the biosorbent concentration favours the heavy metal biosorption because of the increase in surface area (Esposito *et al.* 2001).

The rate of biosorption was high during the beginning and gradually decreased, which might be attributed by the availability of the metal binding sites (Verma *et al.* 2006). The choice of biosorbent depends on their ability to quick metal adsorption, easy desorption and their reusability. Of them, desorption and reusability are the most important factors as the adsorbents can be reused to reduce the material cost. The HCl helps the desorption of chromium by affecting the interaction forces between the heavy metal and the binding sites of the adsorbent.

5. Conclusion

Heavy metal contamination in the freshwater ecosystem poses serious risk to the living systems than that of the heavy metal itself. The ability of fungal resistance against the heavy metal offers a beneficial tool for the monitoring and bioremediation of the heavy metal from the environment. In the present study, the chromium biosorption ability of the dead fungal biomass of *Trichoderma* sp. BSCR02 was evaluated. The biosorption efficiency of the fungal biosorbent was optimized and observed that 1.4 mg/mL of adsorbent was found to be optimum for the biosorption of 200 mg/L of chromium with the initial pH 5 at 35°C during 150 min of contact time. The fungal adsorbent can be reused up to five times with no significant decrease in biosorption efficiency. The findings of the present investigation revealed the possibility of using dead fungal biomass as adsorbent for the removal of chromium ions from the aqueous systems.

Conflict of interest

There is no conflict of interest.

References

Aranda-García E, Morales-Barrera L, Pineda-Camacho G, Cristiani-Urbina E. 2014. Effect of pH, ionic strength, and background electrolytes on Cr(VI) and total chromium removal by acorn shell of *Quercus crassipes* Humb. & Bonpl. *Environ Monit Assess* 186:6207–21. http://dx.doi.org/10.1007/s10661-014-3849-8.. PMID: 24880725.

Aravindhan R, Fathima A, Selvamurugan M, Rao JR, Balachandran UN. 2012. Adsorption, desorption, and kinetic study on Cr(III) removal from aqueous solution using *Bacillus subtilis* biomass. *Clean Technol Environ Policy* 14:727–35.

Bakri Y, Masson Magali, Thonart Philippe. 2010. Isolation and identification of two new fungal strains for xylanase production. *Appl Biochem Biotechnol* 162: 1626–34.

Barman SC, Kisku GC, Salve PR, Misra D, Sahu RK, Ramteke PW, Bhargava SK. 2001. Assessment of industrial effluent and its impact on soil and plants. *J Environ Biol* 22(4):251–6.

Doğan M, Abak H, Alkan M. 2009. Adsorption of methylene blue onto hazelnut shell: kinetics, mechanism and activation parameters. *J Hazard Mater* 164(1): 172–81.

Eisler R. 1986. Chromium Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. Laurel, MD: Fish and Wildlife Service Biological Report 85. pp. 1–6.

Esposito A, Pagnanelli F, Lodi A, Solisio C, Vegliò F. 2001. Biosorption of heavy metals by *Sphaerotilus natans*: an equilibrium study at different pH and biomass concentrations. *Hydrometallurgy* 60:129–41.

Gochev Velizar K, Velkova Zdravka I, Stoytcheva Margarita S. 2010. Hexavalent chromium removal by waste mycelium. *J Serbian Chem Soc* 75:551–64.

Goyal N, Banerjee UC. 2003. Comparative studies on the microbial adsorption of heavy metals. *Adv Environ Res* 7:311–9.

Gulay B, Sema B, Yakup AM. 2003. Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor*. *J Hazard Mater B* 101:285–300.

Hiney M, Dawson MT, Heery DM, Smith PR, Gannon F, Powell R. 1992. DNA probe for *Aeromonas salmonicida*. *Appl Environ Microbiol* 58:1039–42.

Iqbal M, Edvean RGJ. 2004. Biosorption of lead, copper and zinc ions on loofa sponge immobilized biomass of *Phanerochaete chrysosporium*. *Miner Eng* 17:217.

Khezami L, Capart R. 2005. Removal of chromium(VI) from aqueous solution by activated carbons: kinetic and equilibrium studies. *J Hazard Mater* B123:223–31.

Leung WC, Wong MF, Chua H, Lo W, Yu PHF, Leung CK. 2000. Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Wat Sci Technol* 12:233–40.

Nasseri S, Mazaheri AM, Noori SM, Rostami KH, Shariat M, Nadafi K. 2002. Chromium removal from tanning effluent using biomass of *Aspergillus oryzae*. *Pak J Biol Sci* 5(10):1056–9.

Netzahuatl-Muñoz AR, Cristiani-Urbina MdC, Cristiani-Urbina E. 2015. Chromium biosorption from Cr(VI) aqueous solutions by *Cupressus lusitanica* Bark: kinetics, equilibrium and thermodynamic studies. *PLoS One* 10(9), e0137086. http://dx.doi.org/10.1371/journal.pone.0137086.

Norzatulakma MK. 2010. Treatment of industrial wastewater at Gebeng area using *Eichornia crassipes* sp. (Water Hyacinth), *Pistia stratiotes* sp. (Water Lettuce) and *Salvinia molesta* sp. (Giant Salvinia). *Universiti Malaysia Pahang* 7(12):3802–7.

Pandey A, Banerjee D. 2011. Biosorption of cadmium (II) using discarded biomass of *Aspergillus aculeatus* DBF9. *Terrestrial Aquatic Environ Toxicol* 6(11):8–13.

Pandit R, Patel B, Kunjadia P, Nagee A. 2013. Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi Ankleshwar, Gujarat. *Inter J Environ Sci* 3(5):1689–99.

Puentes-Cárdenas IJ, Pedroza-Rodríguez AM, Navarrete-López M, Villegas-Garrido TL, Cristiani-Urbina E. 2012. Biosorption of trivalent chromium from aqueous solutions by *Pleurotus ostreatus* biomass. *Environ Eng Manag J* 11: 1741–52.

Rakhunde R, Deshpande L, Juneja HD. 2012. Chemical speciation of chromium in water: a review. *Crit Rev Environ Sci Technol* 42(7):776–810.

Saha B, Orvig C. 2010. Biosorbents for hexavalent chromium elimination from industrial and municipal effluents. *Coord Chem Rev* 254:2959–72.

Samuel MS, Abigail MEA, Ramalingam C. 2015. Biosorption of Cr(VI) by *Ceratocystis paradoxa* MSR2 using isotherm modelling, kinetic study and optimization of batch parameters using response surface methodology. *PLoS One* 10(3), e0118999.

Santhi R, Guru V. 2014. Biosorption of hexavalent chromium using *Aspergillus niger* dead biomass and its optimization studies. *Int J Curr Microbiol Appl Sci* 3(10): 669–78.

Sardrood BP, Goltapeh EM, Varma A. 2013. An Introduction to Bioremediation Fungi as Bioremediators. New York: Springer, New York. pp. 3–27.

Sen M, Ghosh Dastidar M. 2010. Chromium removal using various biosorbents. *Iran J Environ Health Sci Eng* 7(3):182–90.

Sethuraman P, Balasubramanian N. 2010. Removal of Cr(VI) from aqueous solution using *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*. *Int J Eng Sci Technol* 2(6):1811–25.

Shankar C, Sridevi D, Joonhong P, Michael D, Kaliannan T. 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J Hazard Mater* 146:270–7.

Singh Anuradha, Shahid Mohammad, Srivastava Mukesh. 2014. Phylogenetic relationship of *Trichoderma asperellum* Tasp/8940 using internal transcribed spacer (ITS) sequences. *Int J Adv Res* 2(3):979–86.

Stasinakis AS, Thomaidis NS, Mamais D, Karivali M, Lekkas TD. 2003. Chromium species behavior in the activated sludge process. *Chemosphere* 52:1059–67.

Sugasini A, Rajagopal K. 2015. Hexavalent chromium removal from aqueous solution using *Trichoderma viride*. *Int J Pharma Biosci* 6(1):485–95. (B).
Sujaul IM, Hossain MA, Nasly MA, Sobahan MA. 2013. Effect of industrial pollution on the spatial variation of surface water quality. *Am J Environ Sci* 9(2):120.
Tian-Wei T, Hu B, Haijia S. 2004. Adsorption of Ni^{2+} on amine-modified mycelium of *Pencillium chrysogenum*. *Enzyme Microb Technol* 35:508–13.
Verma A, Chakraborty S, Basu JK. 2006. Adsorption study of hexavalent chromium using tamarind hull-based adsorbents. *Sep Purifi Tech* 50(3):336–41.
Viamajala S, Peyton BM, Sani RK, Apel WA, Petersen JN. 2004. Toxic effects of chromium (VI) on anaerobic and aerobic growth of *Shewanella oneidensis* MR-1. *Biotechnol Prog* 20:87–95.
Vijayaraghavan K, Palanivelu K, Velan M. 2006. Biosorption of copper (II) and cobalt (II) from aqueous solutions by crab shell particles. *Bioresour Technol* 97(12): 1411–9.
Volesky B. 1990. In: Volesky B (Ed.). *Biosorption and Biosorbents in Biosorption of Heavy Metals*. Boca Raton: CRC Press Inc, Boca Raton.
Yan G, Viraraghavan T. 2003. Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. *Water Res* 37:4486–96.
Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 7:203–14.