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Exploring Bioremediation Approach on Removal of Hexavalent Chromium (Cr^{6+}) in Synthetic Medium using Active and Inactive Fungal Bioadsorbents

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A B S T R A C T

Removal of hexavalent chromium was studied in an aqueous medium using fungal biomass as bioadsorbents. *Aspergillus flavus* (S12) was used to investigate the removal of hexavalent chromium in aqueous medium. The present study has been focused on the removal of hexavalent chromium(VI) using a fungal biomass as adsorbents for both active and inactive biomass conditions. For optimization, varying batch study was carried out with varying inoculum (0.5, 1, 1.5, 2 and 2.5%), pH (3, 5, 7, 9 and 11) and temperature (25, 30, 35, 40 and 45°C). In-situ, lab scale column studies were approached for the removal of chromium. Cr(VI) removal rate was attained in aqueous medium using active biomass which was 89.67% and inactive biomass showed 94.55% using. The optimum inoculum for active biomass is 1.5%, inactive biomass is 0.5%, the optimum pH for both active and inactive biomass is 3.0, the optimum temperature is 35°C. The characterization of fungal biomass using SEM-EDX analysis confirmed the removal of Cr((VI) in aqueous medium. FTIR analysis showed that amine, alcohol, alkene and carbon dioxide represent the before and after treatment chromium(VI) in active and inactive fungal biomass (adsorbents).

Introduction

Heavy metal ions are the most common contaminants, they are non-degradable and highly toxic in nature, which leads to a soil and water pollution (Ayangbenro and Babalola, 2017; Kahlon *et al.,* 2018). Heavy metal ions can be detected by using many techniques such as the three main groups: optical detection techniques, electrochemical techniques and spectroscopic detection techniques. In the spectroscopic method, atomic absorption spectroscopy, inductively coupled plasma mass spectrometry and atomic emission spectroscopy methods are used to detect heavy metal ion concentrations (Lebedev *et al.,* 2003). Among the heavy metal ion detection techniques, the adsorption method is a better one because of its used dilute solutions toxic substances, its high removal efficiency, low cost, comparatively simple method of design and strong

feasible (Wang *et al.,* 2013). Heavy metal ions are detected by using suitable methodological procedures. Quantify the toxic effect of soil and water pollution from heavy metal ions (Ahmad *et al.,* 2019). In chemical industries, the main issue is that widespread contaminants lead to heavy metal ions, namely cadmium, chromium, titanium, tin, silver, platinum, vanadium, nickel and copper (Ali *et al.,* 2019; Jaishankar *et al.,* 2014; Kim *et al.,* 2019). All the heavy metals can act as a carcinogenic agent in milling, anodizing-cleaning, conversion coating, electrolyte depositing (Carolin *et al.,* 2017). Bioremediation is a technique that represents how microbes are to remediate toxic substances in biological ways in the ecosystem (Siddiquee *et al.,* 2015). The process was carried out by using microorganisms such as bacteria, fungi, toxicity hazardous materials to human health, which defined as a process involving biologically degraded materials.

The process is enhanced by adding a nutrient, an electron acceptor. This method is a low-cost and low-technology technique (Su *et al.,* 2014). Microbes have the capability of bioremediating heavy metals, bioflocculants, biosorptive abilities, and adsorbent act as a main role. Microbial biomass to remediate pollution of soil and water contaminants. Physical, bioreactor configuration and chemical methods are used to enhance the biosorption from the microbial cells (Ayangbenro *et al.,* 2017).

Acinetobacter sp. and *Arthrobacter* sp. bacterial consortium to remove the 78% chromium in the 16mg/L metal concentration (Puyen, *et al.,* 2012). Remediation of Cr (VI) has been reported under laboratory conditions for *Pseudomonas* sp. (Tarekegn *et al.,* 2020). Fungal organism biomass yields were used mostly in large-scale industrial fermentation processes (Xue *et al.,* 2012). *Aspergillus* sp. is used in the production of glucose isomerase, lipases and glucanases. Active biomass, when compared to inactive biomass, produces a large amount of yield and is easy to remove Cr(VI) from waste water treatment. Inactive biomass is good source for biological material for Cr(VI) removal (Park *et al.,* 2014). In the present study, we demonstrate the hexavalent chromium(VI) is converted into trivalent chromium by using fungal biomass for both active and inactive conditions. Screening, optimization of varying inoculum, pH and temperature studies were carried out. Lab-scale column studies for Cr(VI) removal and characterization analysis were carried out SEM-EDX and FTIR instrumentation.

Materials and Methods

Chromium tolerant fungi from tannery industry

The soil sample and tannery effluent contaminated with chromium was collected from the prime tannery industry, located in Ranipet District of Tamil Nadu, India. Spread plate technique was employed to isolated indigenous fungal strains using Sabouraud dextrose agar. The chromium contaminated fungal strains were isolated using spread plate technique, and the fungi were screened using spot plate assay method. The primary screening was done in Sabouraud dextrose agar medium, and the pH was adjusted to 5.0. Chromium concentrations ranging from 200 to 1000 mg/L were incorporated in SDA medium with different concentrations. The medium was autoclaved 121°C and poured. The solidified medium was kept in aseptic condition without and disturbances. The individual fungal strains isolated were spotted on SDA medium and the plates were incubated at 28-30°C for 7 days. After the incubation all the plates were observed for the growth of fungal strains, and the results were noted (Gautam *et al.,* 2021). From the preliminary screening, based on the morphological characteristics and growth of the organisms, four Cr(VI) tolerant fungi were investigated using 100 ml of aqueous medium contains 10 mg/L of Cr(VI) and 1% of active biomass. Similarly, in another set of experiments, instead of active biomass, inactive biomass was inoculated. Then all the conical flasks were kept in a rotary shaker at 28-30 °C in 120 rpm. Every 10 minutes, 2ml of sample were collected from all the conical flasks. The reduction of Cr(VI) was determined, and Cr(III) was also noted in DPC assay method at 540 nm by using UV-Vis Spectrophotometer model (Srivastava *et al.,* 2008).

Preparation of fungal adsorbents

Aspergillus flavus (S12) strain suspensions of spores were collected from stock slants, inoculated on fresh Potato Dextrose Agar (PDA) slant, and incubated at 28-30°C for a week until the fresh spores grew.

The fresh suspension spores were inoculated into 100 ml of Potato Dextrose Broth (PDB), growth medium and then incubated in a rotary shaker at 120 rpm for 28 – 30°C for 3 days. Mycelial pellets (fungal biomass) of similar size were harvested, washed with distilled water five times, and then squeezed to drain out the excess water (Ramrakhiani *et al.,* 2011).

Effect of varying inoculum concentrations of active and inactive fungal biomass on Cr(VI) removal in aqueous medium

Aqueous medium of 100 ml was prepared using different concentrations of active biomass (0.5, 1, 1.5, 2 and 2.5%) and inactive biomass (0.5, 1, 1.5, 2 and 2.5%) with the addition of 10 mg/L of Cr(VI) to study the effect of varying inoculum concentrations of biomass on Cr(VI) reduction by active and inactive fungal biomass.

The *Aspergillus flavus* (S12) strain was inoculated with active and inactive fungal biomass. Then all the conical flasks were kept in a rotary shaker at 28-30 °C at 120 rpm. Every 10 minutes interval, 2ml of sample were collected for the reduction of Cr(VI) and Cr(III), as noted in the DPC assay method at 540 nm by using UV-Vis Spectrophotometer (Ying *et al.,* 2013).

Effect of varying pH on Cr(VI) reduction by active and inactive fungal biomass

The effect of varying pH (pH 3, 5, 7, 9 and 11) studied 100 ml of aqueous medium contains 1% of *Aspergillus flavus* (S12) active biomass and varying pH (pH 3, 5, 7, 9 and 11) of 0.5% *Aspergillus flavus* (S12) of inactive biomass with addition of 10 mg/L of Cr(VI) to study the effect of varying pH on Cr(VI) reduction by active and inactive biomass. Then all the conical flasks were kept in a rotary shaker at 28-30°C in 120 rpm. Every 10 minutes interval, 2ml of sample were collected from all the conical flasks. The reduction of Cr(VI) was determined, and Cr(III) also noted in DPC assay method at 540 nm by using UV-Vis Spectrophotometer (Qian *et al.,* 2017).

Effect of varying temperature on Cr(VI) reduction by active and inactive fungal biomass

The effects of varying temperatures were prepared in an aqueous medium 100 ml using varying temperatures (25, 30, 35, 40 and 45°C) of 1% active biomass (*Aspergillus flavus* (S12)) and various temperatures (25, 30, 35, 40 and 45°C) of 0.5% inactive biomass (*Aspergillus flavus* (S12)) with the addition of 10 mg/L of Cr(VI) to study the effect of various temperatures on Cr(VI) reduction by active and inactive biomass. Then all the conical flasks were kept in a rotary shaker at 28-30 °C in 120 rpm. Every 10 minutes interval, 2ml of sample were collected from all the conical flasks. The reduction of Cr(VI) was determined, and Cr(III) was also noted in the DPC assay method at 540 nm by using UV-Vis Spectrophotometer (Sheng *et al.,* 2017).

Removal of Cr(VI) in lab-scale column enriched with active and inactive fungal biomass

Removal of Cr(VI) from synthetic water was carried out through a column set up containing biomass enriched with selected fungal strain of *Aspergillus flavus* (S12). The set-up was made under laboratory conditions. The glass columns, approximately 30 cm in length and 10 cm in diameter were used in this Cr(VI) removal study. Prior to use, each column was washed 4-5 times with absolute alcohol (99.9%).

Each column was packed with approximately 2 kg of active biomass and inactive biomass, respectively, under aseptic conditions and closed tightly with holed caps. The column with each hole was connected to silicon tubes. The tube from the top of the column was inserted into the synthetic water Cr(VI) collection vessel, and the tube from the bottom was connected to the reservoir containing treated water. There are two sets up of glass column treatment named as Treatment – 1 and Treatment - 2 was carried out. To remove Cr(VI), an appropriate protocol was made and applied in each treatment column, as follows:

Treatment 1: Column packed with active fungal biomass of *A. flavus* (S12)
Treatment 2: Column packed with inactive fungal biomass of *A. flavus* (S12)

The synthetic water with Cr(VI) about 10 mg/l was taken in sterile flasks, and they were kept on the platform undisturbed. The Cr(VI) enriched water from each flask was passed in to the glass column containing active and inactive biomass respectively with the help of silicon tube till the Cr(VI) was completely reduced. Every 10 minutes interval, 2ml of sample were collected from all the conical flasks. The reduction of Cr(VI) was determined, and Cr(III) was also noted in the DPC assay method at 540 nm by using a UV-Vis Spectrophotometer (Ayyasamy *et al.,* 2009).

Characterization of fungal biomass with SEM-EDX and FTIR analysis

SEM-EDX (Scanning Electron Microscope) analysis purified bioflocculant morphology was determined using

a model of EVO 18, Carl Zeiss Microscopy GmbH Germany, resolution at 3nm; magnification about 1× to 1000000×.The treated and untreated mycelial pellets of morphology was analyzed using SEM- EDX (Ren *et al.,* 2018; Silvia *et al.,* 2023). Treated and untreated mycelial pellets of functional groups and binding sites were examined using a Fourier Transform infrared (FTIR) spectrophotometer with a spectral range about 400-4000 cm^{-1}. The instrument used was the Shimadzu IR Spirit. After the study, the treated and untreated mycelial pellets of fungal biomass were collected, washed with deionized water, and dried in an oven at 60°C. The collected pellets were mixed with KBr pellets for analysis (Rafi *et al.,* 2017; Singh *et al.,* 2010).

Results and Discussion

Fungal strains from soil and tannery effluent samples

The study revealed that 12 fungal species were isolated from four different sites of soil and tannery effluent from chromium-contaminated sites. The fungal strains were confirmed based on the fungal morphological characters using SDA medium and LPCB wet mount staining. In Tamil Nadu, highly chromium contaminated areas, one of the District is Ranipet, as depicted in figure 1.

Screening of chromium(VI) tolerant fungal strains

Primary screening to find out chromium(VI) tolerant fungal strains based on the growth of the organisms ranged from 200 to 1000 mg/L of Cr(VI). Among 12 isolates, based on the growth of the organisms, 4 isolates (S3, S10, S11 and S12) were selected for further studies. The Cr(VI) concentrations growth were noticed and depicted in table 1.

The results revealed that among the 12 fungal isolates, four strains were only selected for secondary screening of Cr(VI) tolerant fungal strains. In this experiment, based on the preliminary screening, four different potential strains (S3, S10, S11 and S12) were selected. The percentage removal rate of Cr(VI) was assessed using the standard graph (Fig. 2). Percentage removal in the screening study showed 70.6, 72.8, 71.2 and 92.6% using active biomass of S3, S10, S11 and S12 respectively. Similarly, the removal of Cr(VI) was recorded about 66.2, 72.9, 78.4 and 98.01 using inactive biomass of S3, S10, S11 and S12 respectively. The active and inactive biomass, among the four strains, one potential strain (S12) was selected for further studies, and the potential strain *Aspergillus flavus* (S12) was selected based on the Cr(VI) removal rate, as noticed and depicted in figure 3.

Morphology of mycelial pellet

Aspergillus flavus (S12) was harvested by using potato dextrose broth medium in mycelium. The cultivated mycelial pellets were incubated at 28-30 °C for 3 days, and the size of the mycelial pellets is approximately 2.8 mm.

Cr(VI) reduction in aqueous medium using active and inactive fungal biomass

Effect of varying concentrations of active and inactive biomass (0.5, 1, 1.5, 2 and 2.5%) on Cr(VI) reduction was investigated by *Aspergillus flavus (S12)*. The active biomass removal rate of Cr(VI) is 0.5% at 80.57%, 1% at 80.90%, 1.5% at 84.67%, 2% at 83.22% and 2.5% at 76.6%. The inactive biomass shows 0.5% at 97.75%, 1% at 88.20%, 1.5% at 89.44%, 2% at 89.64% and 2.5% at 87.79%. The Cr(VI) removal rate is recorded every 10 minutes for the accumulation of Cr(VI) concentration in active and inactive fungal biomass. Active fungal biomass optimum inoculum is 1.5% and the inactive fungal biomass optimum inoculum is 0.5%, and the results are shown in figure 4.

Cr(VI) reduction in aqueous medium using active and inactive fungal biomass at varying pH

Effects of varying pH (3, 5, 7, 9, and 11) were determined using aqueous medium (100 ml). Separately, 1.5% of active and 0.5% of inactive biomass with the addition of 10 mg/L of Cr(VI) were studied at different pH (3, 5, 7, 9, and 11), and then the flasks were kept in rotary shaker at 28-30 °C in 120 rpm. Every 10 minutes, the Cr(VI) removal rate was noted using the DPC assay method by using UV-Vis spectrophotometer.

The active fungal biomass Cr(VI) removal rate is shown at pH 3, 5, 7, 9, and 11 respectively, at 93.69%, 90.52%, 86.37%, 87.10%, and 86.80%. The inactive fungal biomass represents the pH 3, 5, 7, 9, and 11 Cr(VI) removal rate at 96.80, 94.51, 78.37, 75.08 and 88.92% for respective pH levels. The results of the study are shown in figure 5.

Cr(VI) reduction in aqueous medium using active and inactive fungal biomass at varying temperatures

The potential fungal strain *Aspergillus flavus* (S12) was studied in the presence of varying temperatures (25, 30, 35, 40, and 45 °C) aqueous medium (100 ml). Separately 1.5% of active and 0.5% of inactive biomass with the addition of 10 mg/L of Cr(VI) were studied. The active and inactive fungal biomass Cr(VI) removal rates were noted using the DPC assay method, and the active fungal biomass Cr(VI) removal rates were observed in 25, 30, 35, 40, and 45 °C. The results noted were 93.19, 82.74, 93.60, 81.24 and 84.74% removal of Cr(VI). The inactive fungal biomass showed that the removal rate of Cr(VI) about 79.90, 88.02, 99.80, 72.05 and 79.79% respectively. The optimum temperature for both active and inactive fungal biomass about 35 °C and the results are shown in figure 6.

Removal of Cr(VI) in synthetic medium using lab scale column enriched with active and inactive fungal biomass

Removal of Cr(VI) from synthetic water was carried out through a column set up containing fungal biomass enriched with selected fungal strain of *Aspergillus flavus* (S12). Approximately 2 kg of biomass per column presumptively enriched with fungal biomass, was selected from the for the removal of Cr(VI).

There are two sets of glass column treatments (1 and 2) was made. Each column was packed with approximately 2 kg of active biomass and inactive biomass, respectively, under aseptic conditions and closed tightly with holed caps. The column with each hole was connected to the tubes. The tube from the top of the column was inserted into the synthetic water Cr(VI) collection vessel, and the tube from the bottom was connected to the reservoir containing Cr(VI) treated water. There are two sets of glass column treatment from treatment- 1 and treatment - 2 was made and carried out. After biomass digestion, the presence of Cr(VI) and Cr(III) was analyzed using DPC assay method.

The first set of studies (Column treatment-1) was carried out with active biomass (*Aspergillus flavus* – S12*)* enriched with 10 mg/L of Cr(VI). Initially, the removal of Cr(VI) was less. Every 10 minutes, the efficiency of Cr(VI) removal was increased. After that, the removal of Cr(VI) was higher, and during that particular period, the efficiency of Cr(VI) removal was increased. However, the removal efficiency was satisfactory for both treatments. The second set of studies (Column treatment-2) was carried out with inactive biomass (*Aspergillus flavus* – S12*)* enriched with 10 mg/L of Cr(VI). Initially, the removal of Cr(VI) was less. The removal of Cr(VI) was more significant when the active and inactive biomass across the column containing. Every 10 minutes, 2 ml of sample were collected from all the conical flasks. Then OD value was taken at 540 nm to find the reduction of Cr(VI) and Cr(III) by using a UV-spectrophotometer. The active fungal biomass removal rate of Cr(VI) is 89.67%, and inactive fungal biomass removal rate is 94.55%. After that, the Cr(VI) removal were noted for whole study. The pH and electrical conductivity (EC) of the Cr(VI) synthetic medium were everyday noted, and the results are shown in figure 7.

SEM-EDX characterization of fungal biomass after treatment

SEM-EDX analysis confirmed the presence of chromium spores enriched mycelial pellets (fungal biomass) on the surface *Aspergillus flavus* (S12) Fig. 8 to 11. Both active and inactive biomass conditions with before and after treatment of chromium were confirmed to represents the cell accumulation in fungal biomass. Before treatment of Cr(VI) cells, the undefined structure was indicated, and after treatment of the Cr(VI) enriched cells, sharp, clear images were noticed. The EDX spectra of the *Aspergillus flavus* (S12) spores were shown the before and after treatment with Cr(VI).

The EDX analysis represents before and treatment of Cr of *Aspergillus flavus* (S12) fungal biomass are characteristic EDX peak patterns of Cr, and the before and after treatment of Cr results are shown in figures (active fungal biomass without Cr), figure (active fungal biomass with Cr), figure (inactive fungal biomass without Cr) and figure (inactive fungal biomass with Cr). Overall, the EDX analysis represents before treatment Cr content biomass sorption is very low and the after treatment, Cr biomass sorption is high.

FTIR characterization of fungal biomass after treatment

The FTIR shows (fig. 12) the presence and absence of Cr(VI).

Table.1 Primary screening of Cr(VI) tolerance by spot plate assay

Strain No	Different concentration of Cr(VI)				
	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm
S1	+	+	-	-	-
S2	+	-	-	-	-
S3	+	+	+	+	+
S4	+	+	-	-	-
S5	+	+	-	-	-
S6	+	+	-	-	-
S7	+	+	-	-	-
S8	+	+	-	-	-
S9	+	+	-	-	-
S10	+	+	+	+	+
S11	+	+	+	+	+
S12	+	+	+	+	+

Where +ve Denotes fungal growth, -ve denotes no fungal growth

Fig.1 Generic distribution of fungal species isolated from Cr(VI) contaminated soil and effluent

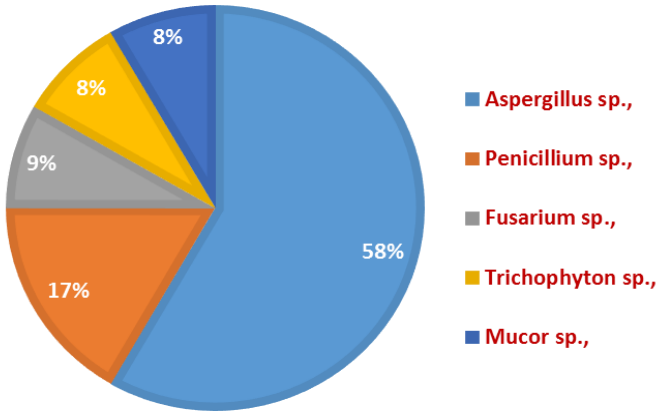
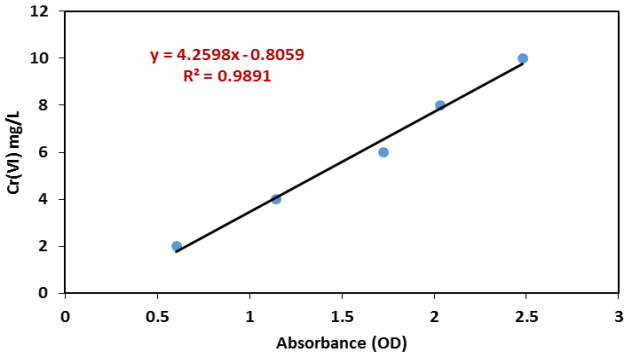


Fig.2 Preparation of Cr(III) and Cr(VI) standard curve

a) Estimation of Cr(VI)



b) Estimation of Cr(III)

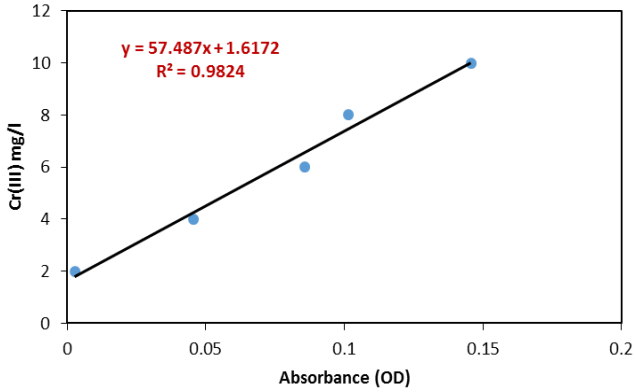
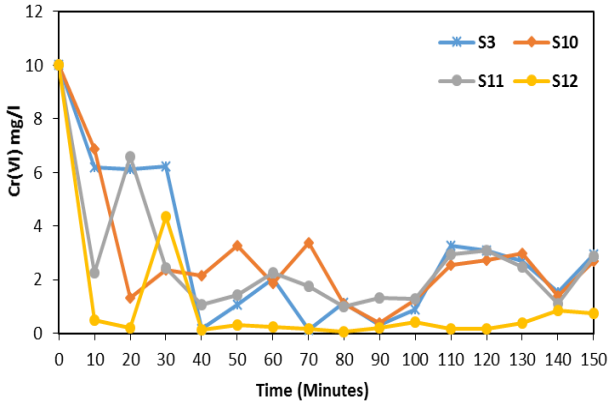
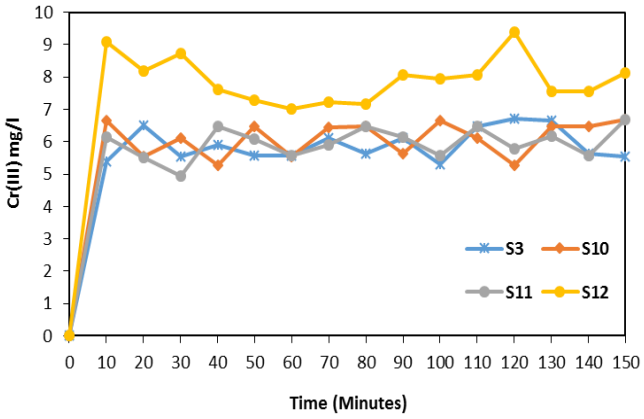


Fig.3 Secondary Screening by broth culture method by active and inactive fungal biomass

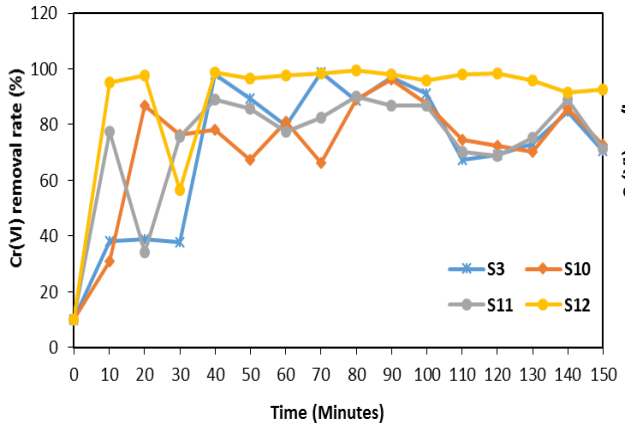
a) Active fungal biomass Cr(VI)



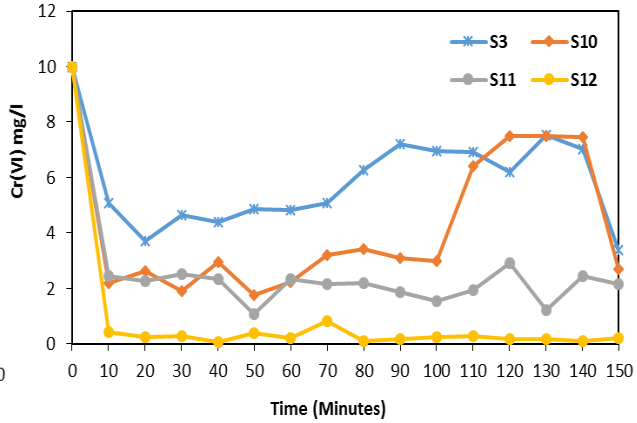
b) Active fungal biomass Cr(III)



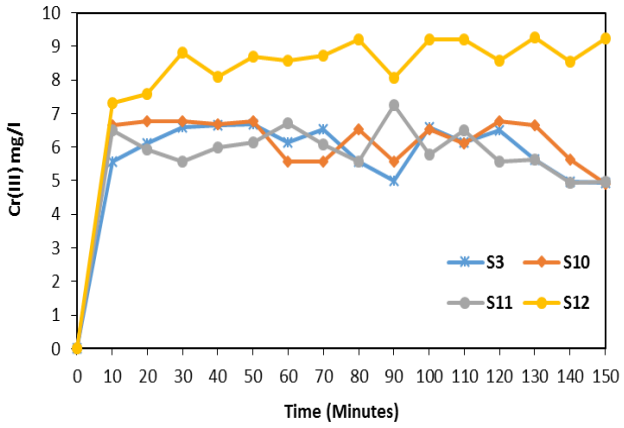
c) Active removal rate of Cr(VI)



d)Inactive fungal biomass Cr(VI)



e) Inactive fungal biomass Cr(III)



f) Inactive removal rate of Cr(VI)

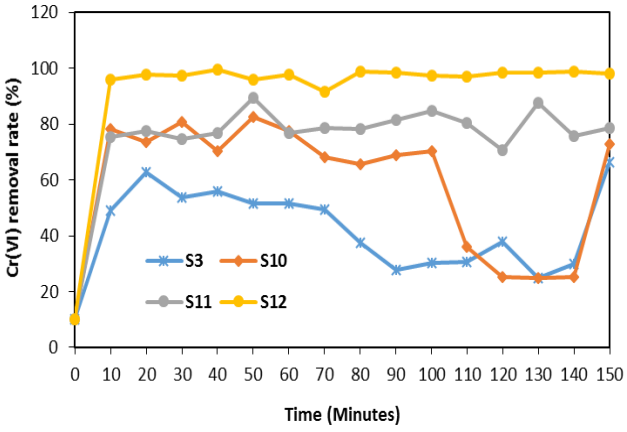
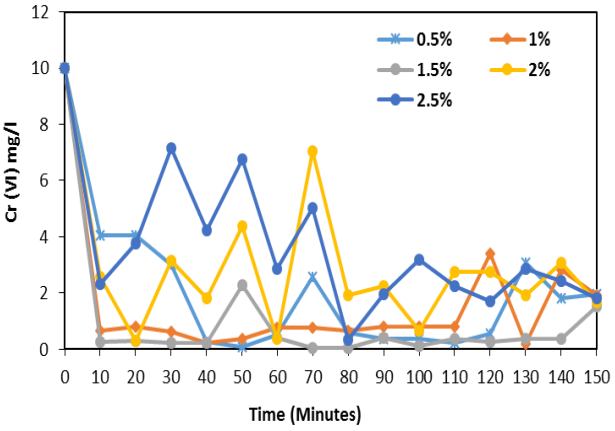
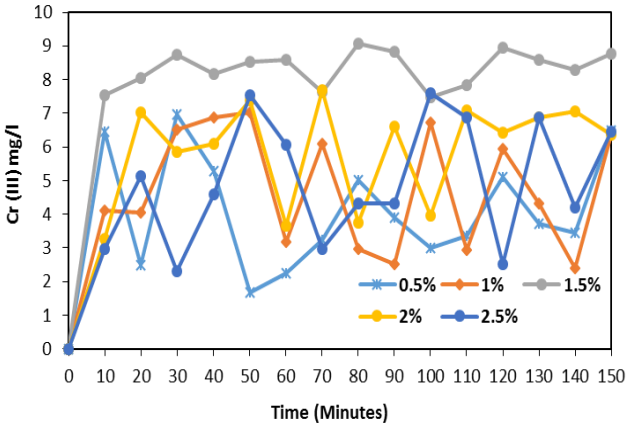


Fig.4 Effect of varying concentration of active and inactive fungal biomass on Cr(VI) reduction

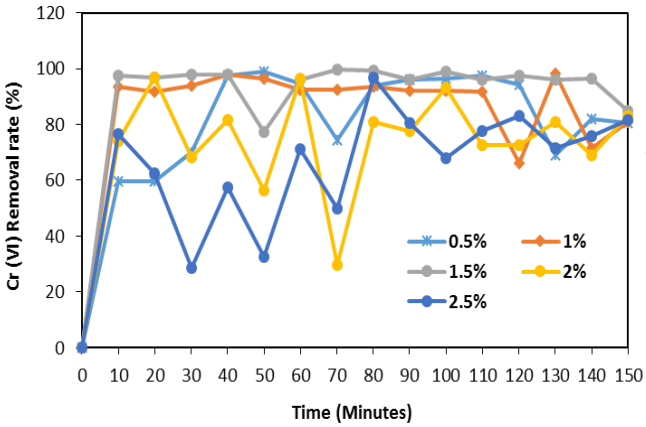
a) Active fungal biomass Cr(VI)



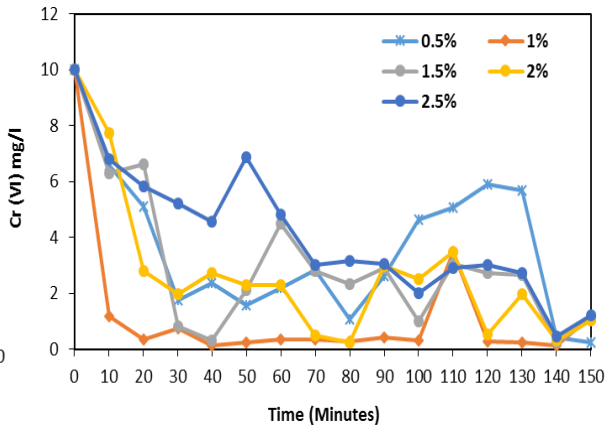
b) Active fungal biomass Cr(III)



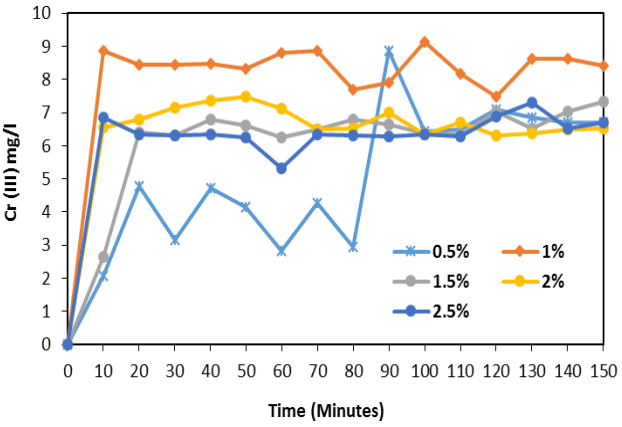
c) Active removal rate of Cr(VI)



d) Inactive fungal biomass Cr(VI)



e) Inactive fungal biomass Cr(III)



f) Inactive removal rate of Cr(VI)

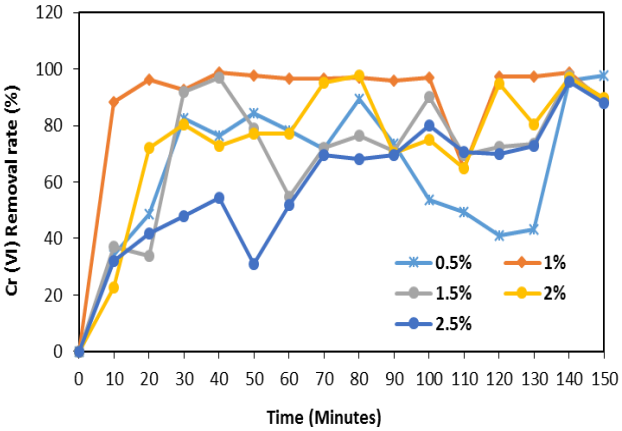
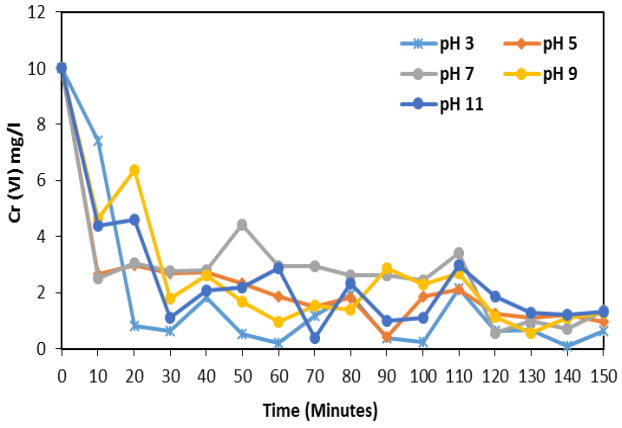
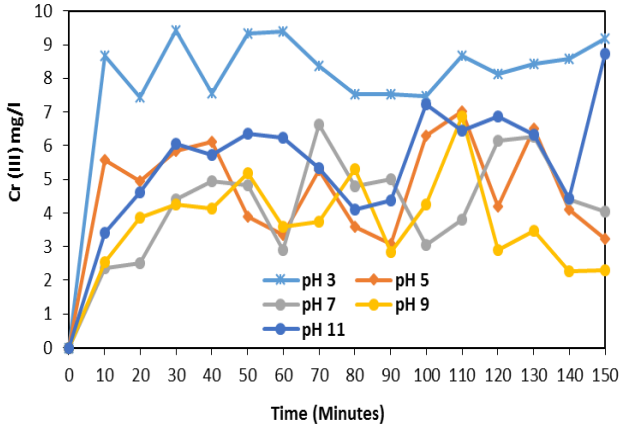


Fig.5 Effect of varying pH on Cr(VI) reduction by active and inactive fungal biomass Cr(VI) reduction

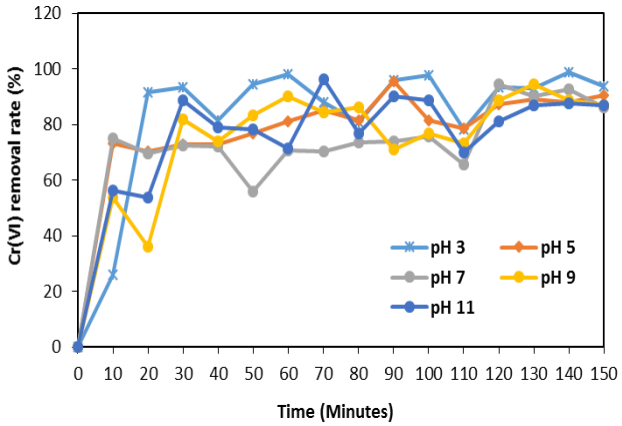
a) Active fungal biomass Cr(VI)



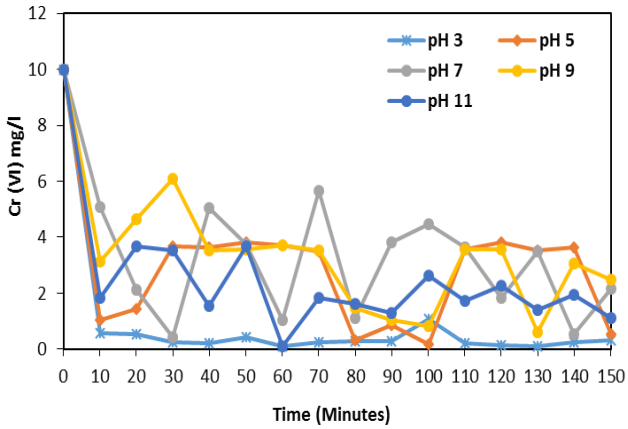
b) Active fungal biomass Cr(III)



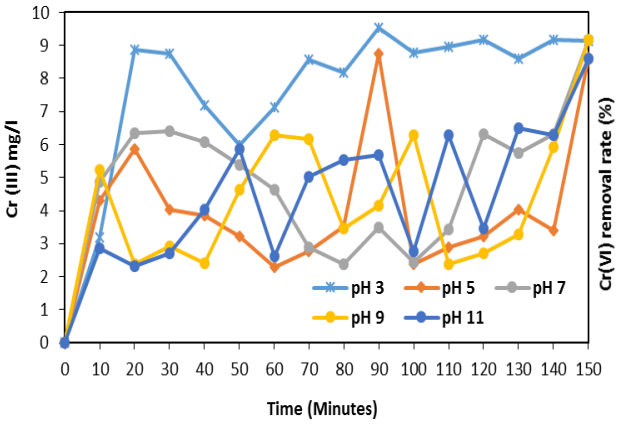
c) Active removal rate of Cr(VI)



d) Inactive fungal biomass Cr(VI)



e) Inactive fungal biomass Cr(III)



f) Inactive removal rate of Cr(VI)

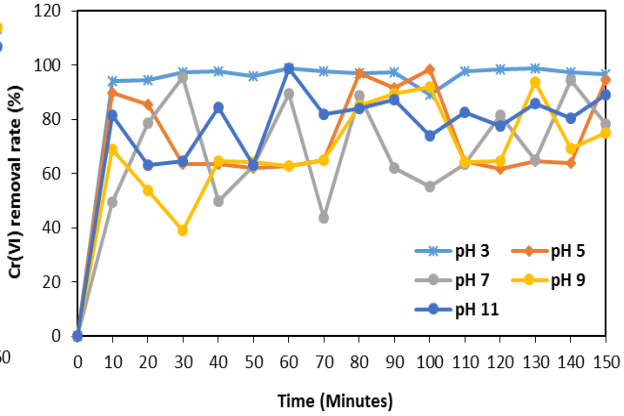


Fig.6 Effect of varying temperature on Cr(VI) reduction by inactive fungal biomass Cr(VI) and reduction

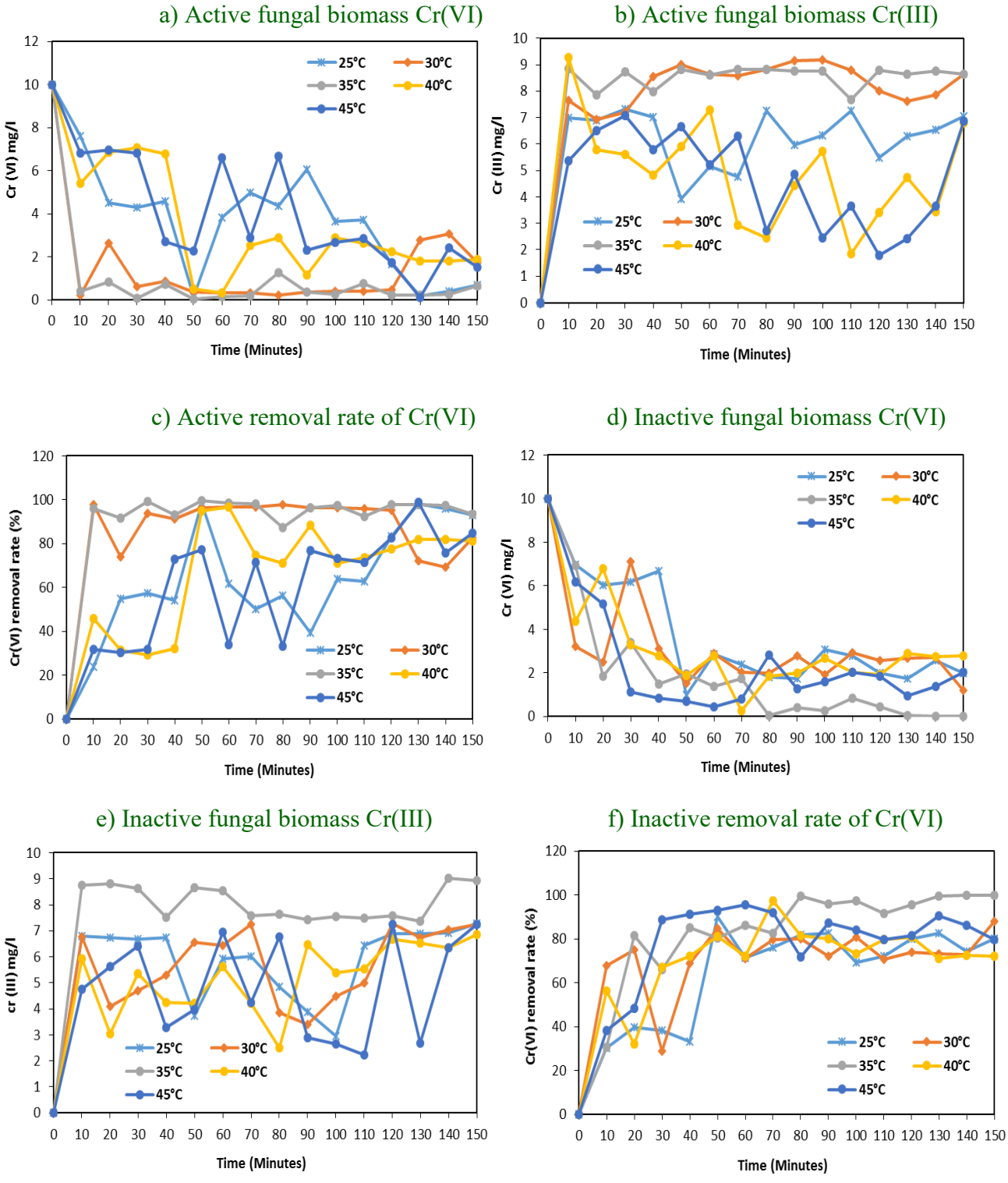
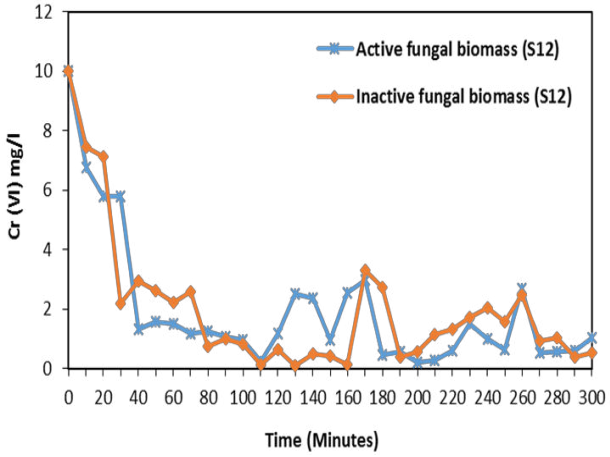
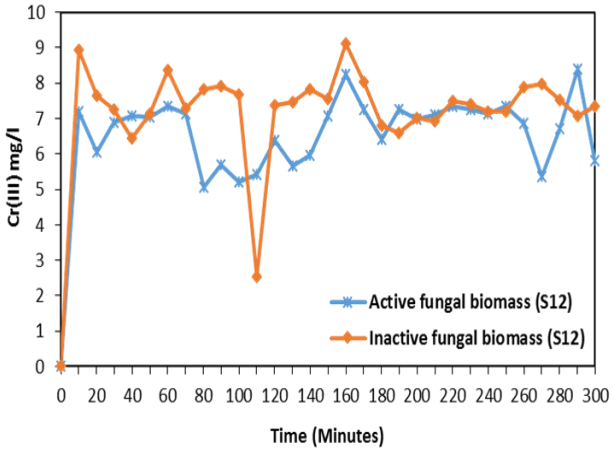


Fig.7 Removal of Cr(VI) in lab scale column enriched with active and inactive fungal biomass

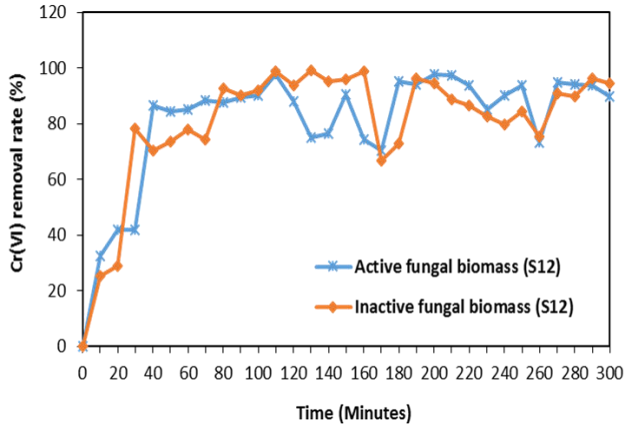
a) Cr(VI)



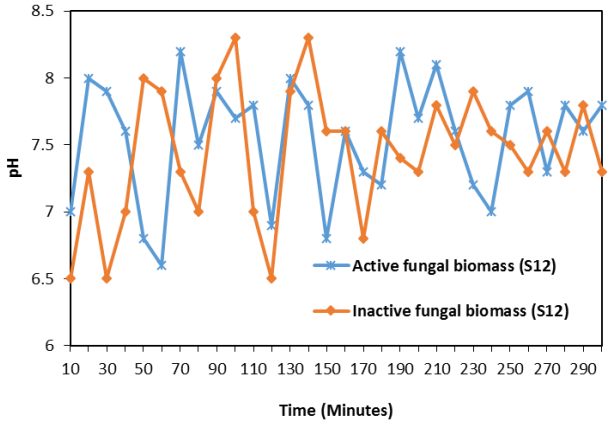
b) Cr(III)



c) Cr(VI) removal rate



d) pH



e) Electrical conductivity (EC)

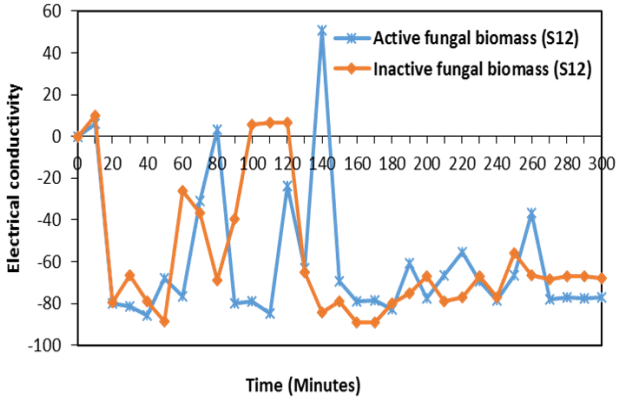
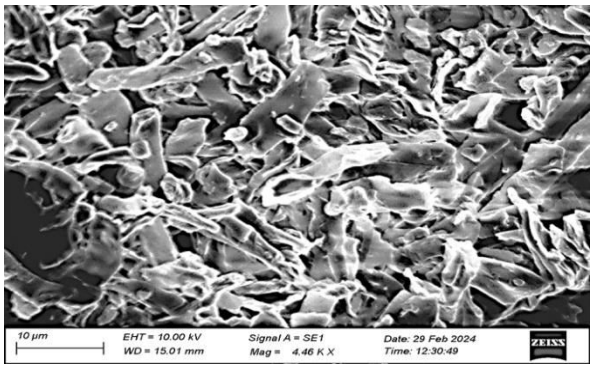
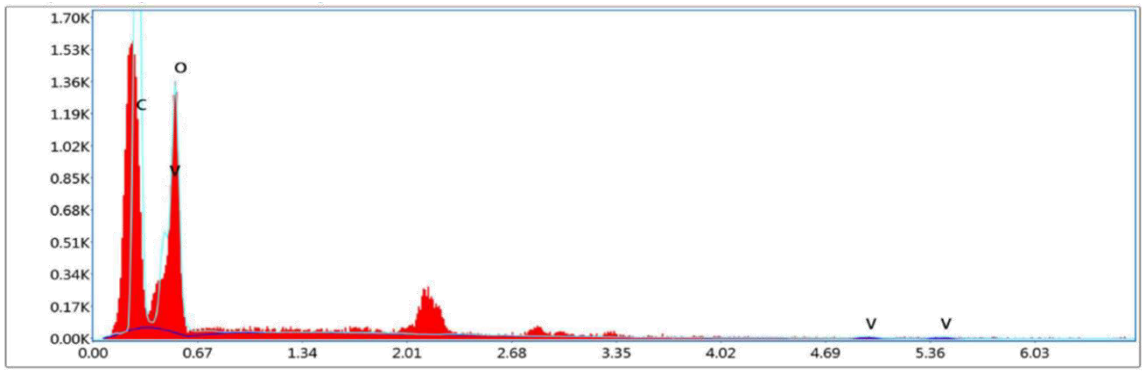


Fig.8 Characterization of the active fungal biomass using SEM and EDAX analysis

(a) SEM image of untreated active fungal biomass (before Cr treatment)



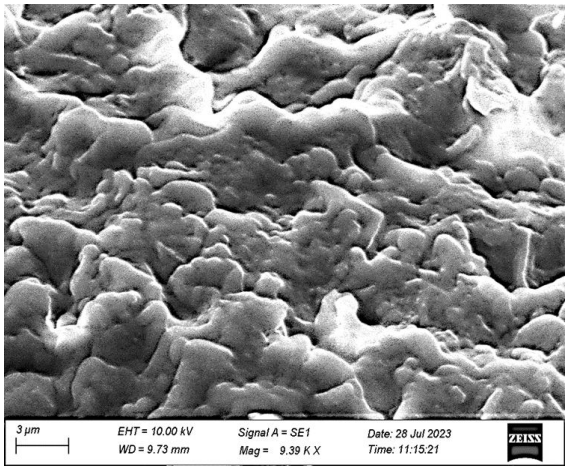
(b) EDX analysis of untreated active fungal biomass (before Cr treatment)



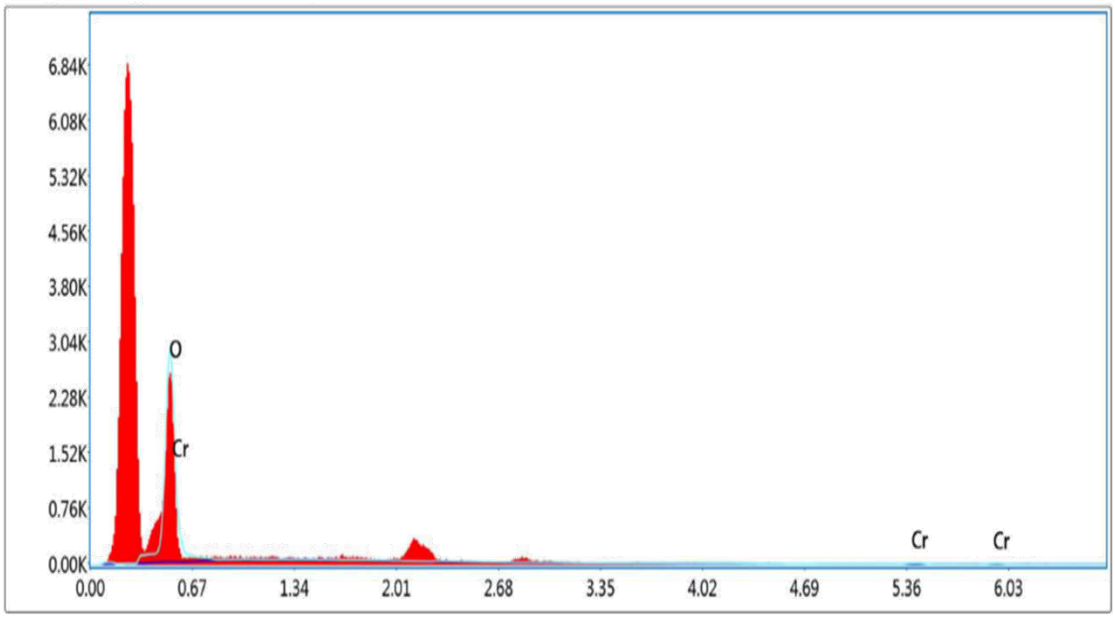
Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	53.02	61.26	201.45	4.83	0.4369	1.0353	0.7959	1.0000
O K	43.61	37.83	106.75	8.78	0.1967	0.9770	0.4615	1.0000
V K	3.37	0.92	1.57	34.02	0.0251	0.6950	1.0073	1.0633

Fig.9 Characterization of the active fungal biomass using SEM and EDAX analysis

(a) SEM image of treated active fungal biomass (after Cr treatment)



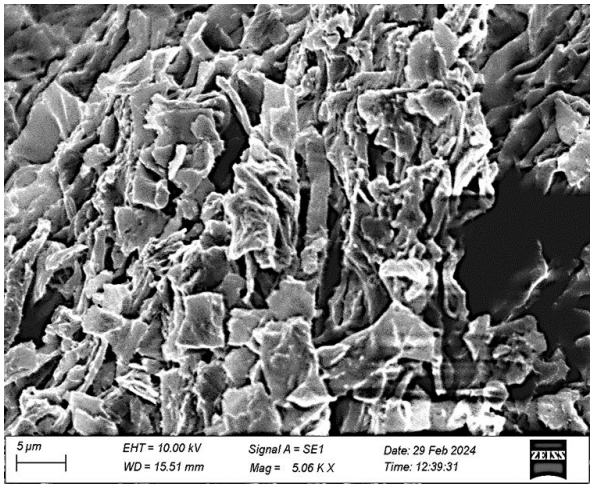
(b) EDX analysis of treated active fungal biomass (after Cr treatment)



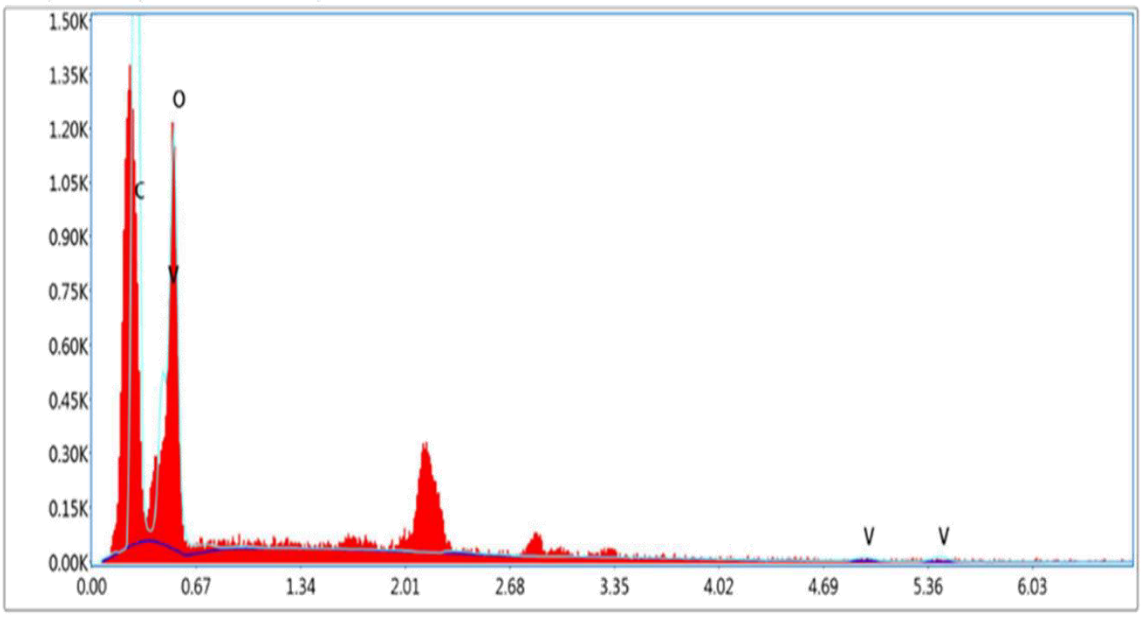
Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	86.49	95.41	235.98	3.35	0.8642	1.0351	0.9654	1.0000
CrK	13.51	4.59	2.55	32.06	0.1040	0.7453	1.0063	1.0264

Fig.10 Characterization of the inactive fungal biomass using SEM and EDAX analysis

(a) SEM image of untreated inactive fungal biomass (before Cr treatment)



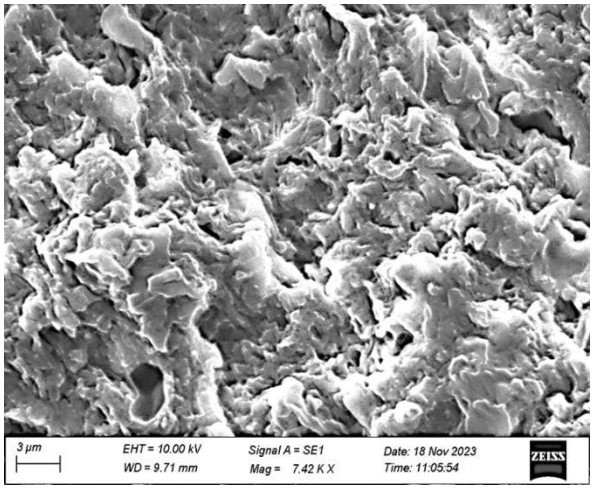
(b) EDX analysis of untreated inactive fungal biomass (before Cr treatment)



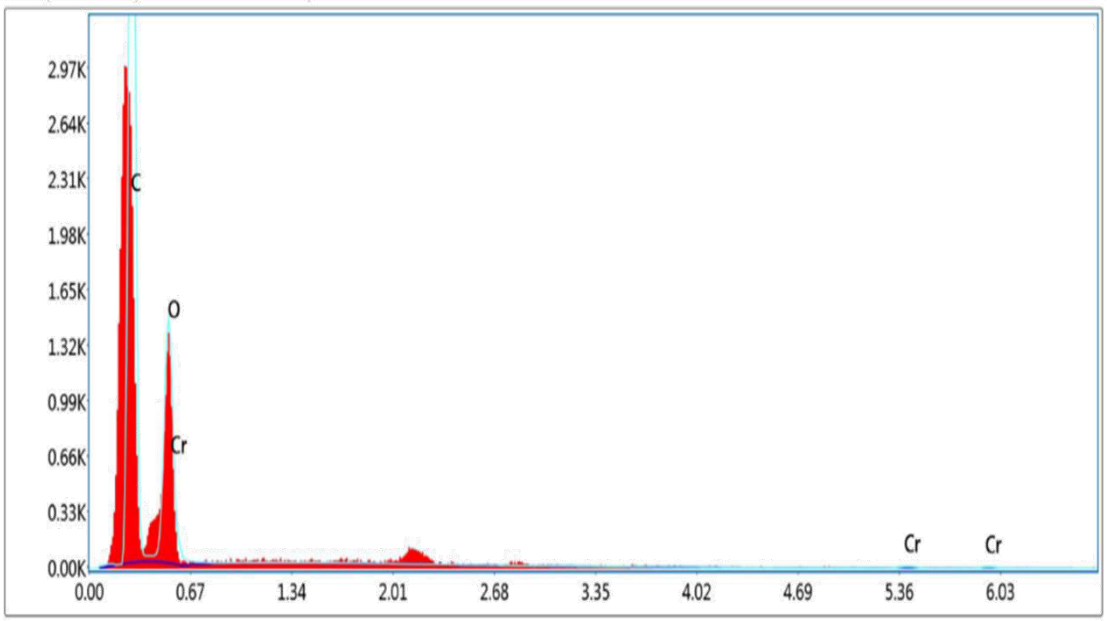
Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	51.87	60.37	171.02	4.97	0.4261	1.0377	0.7917	1.0000
O K	44.09	38.52	94.68	8.88	0.2007	0.9793	0.4648	1.0000
V K	4.05	1.11	1.63	30.53	0.0300	0.6969	1.0081	1.0568

Fig.11 Characterization of the inactive fungal biomass using SEM and EDAX analysis

(a) SEM image of untreated inactive fungal biomass (after Cr treatment)



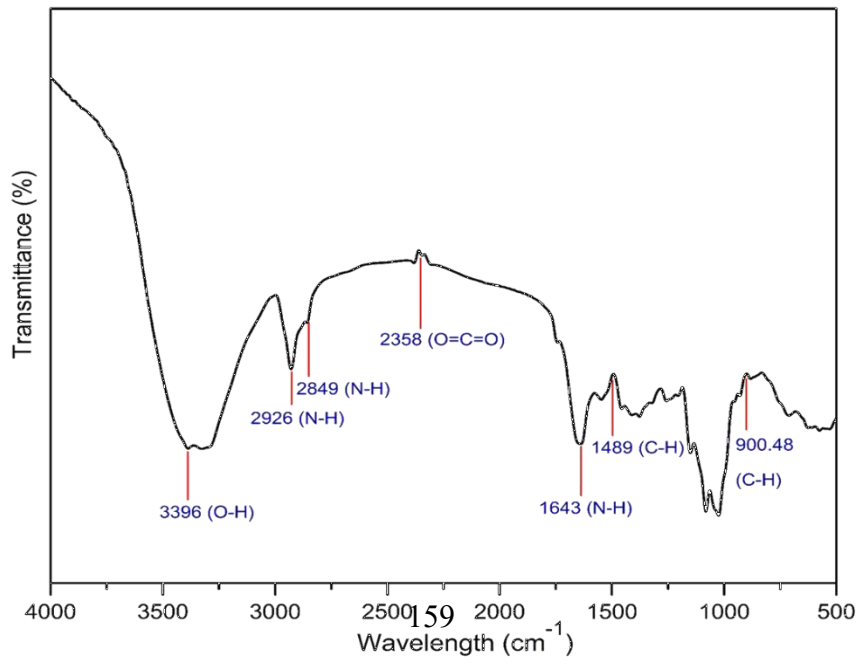
(b) EDX analysis of treated inactive fungal biomass (after Cr treatment)



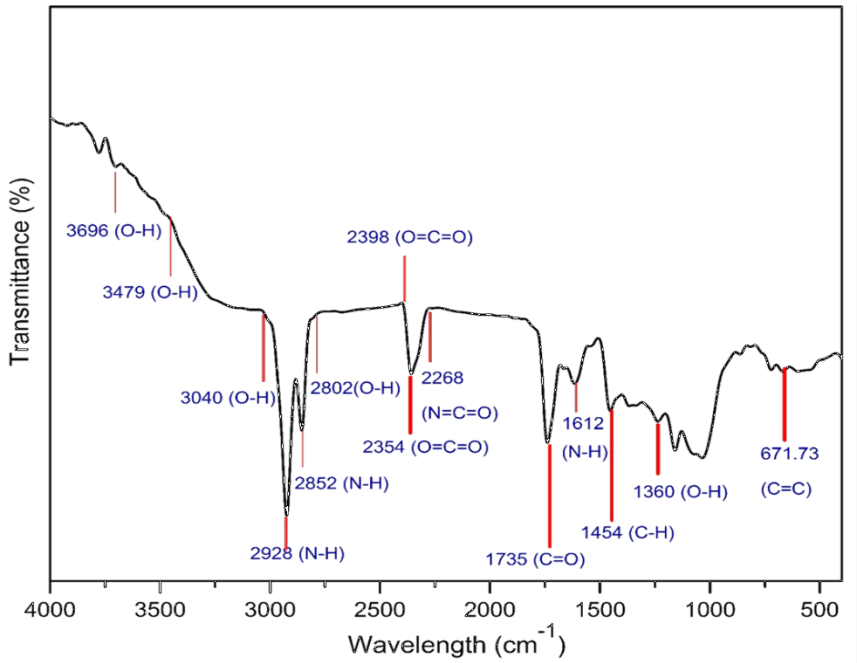
Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	63.30	70.74	377.13	4.30	0.5301	1.0272	0.8153	1.0000
O K	34.06	28.58	113.48	9.35	0.1325	0.9691	0.4015	1.0000
CrK	2.64	0.68	1.54	33.83	0.0201	0.6941	1.0094	1.0879

Fig.12 Characterization of the bioflocculant FTIR analysis

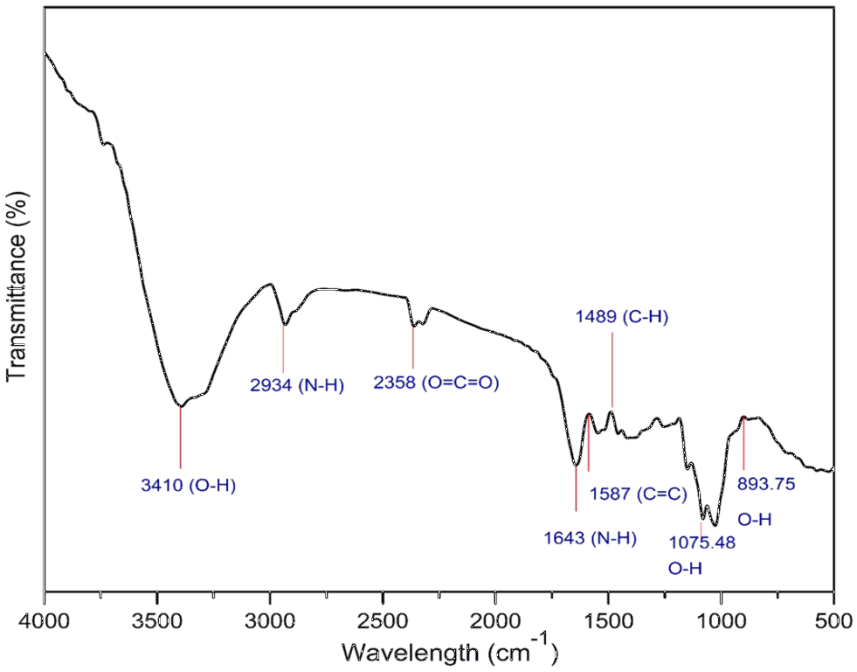
(a) Untreated active fungal biomass (without Cr)



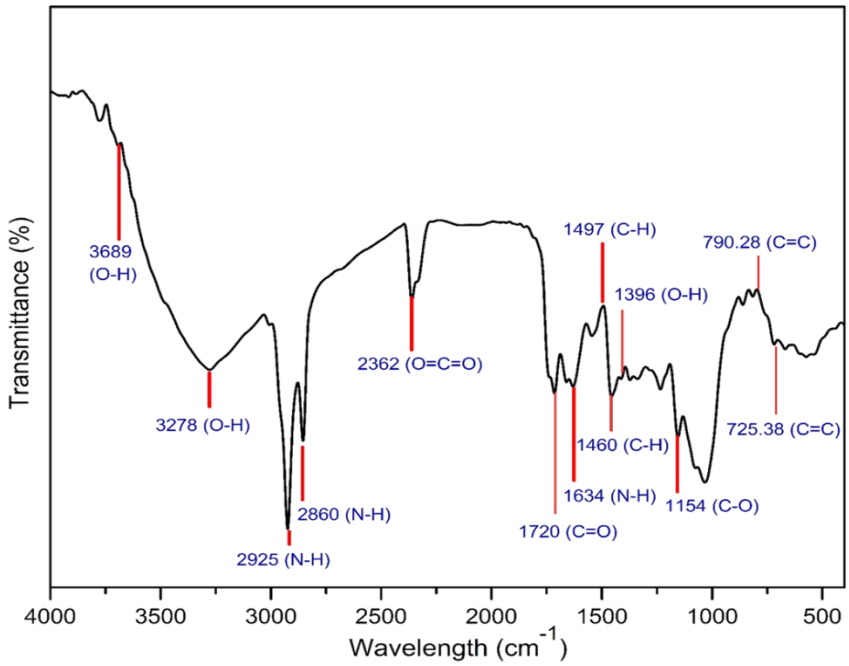
(b) Treated active fungal biomass (with Cr)



(c) Untreated inactive fungal biomass (without Cr)



(d) Treated inactive fungal biomass (with Cr)



Active fungal biomass without Cr sorption were represents the peak like 3396 peak O-H stretching (strong, broad) alcohol, 2926, 2849 and 1643 peak N-H stretching (strong, broad) amine, 2358 peak O=C=O (strong) carbon dioxide, 1489 peak (medium) alkane in the absence of Cr. The presence of Cr active fungal biomass was confirmed by the alcohol, amine, carboxylic acid functional group were shown in 3696 (O-H), 3479 (O-H), 3040 (O-H), 2928 (N-H) 2852(N-H), 2802 (O-H), 2354(O=C=O), 2268 (N=C=O), 1753 (C=O), 1454 (C-H), 1360 (O-H) and 671.73 (C=C). These are all the peaks confirmed the presence of Cr.

Inactive fungal biomass without Cr showed the absence of Cr and confirmed the peak level of FTIR analysis such as, 3410(O-H), 2934 (N-H), 2358 (O=C=O), 1643 (N-H), 1587 (C=C), 1489 (C-H), 1075.48 (O-H) and 893.75 (O-H). The inactive fungal biomass shows a peak at 3689 (O-H), 3278 (O-H), 2925 (N-), 2860 (N-H) 2362 (O=C=O), 1720 (C=O), 1634 (N-H) 1460 (C-H) 1497 (C-H), 1396 (C-H), 1154 (C-O), 790.28 (C=C) and 725.38 (C=C) in the presence of sorption. The presence of Cr was confirmed by the functional peaks in FTIR, which are alcohol, amine, carbon dioxide and alkene.

Reuel *et al.,* 2013 studied *Aspergillus flavus, Aspergillus niger*, and *Aspergillus* sp. based on phenotype, the three isolates were reported. In the present study, out of the 12 isolates, one potential strain (*Aspergillus* sp.) was selected for the production of adsorbents from spore enriched mycelial pellets (fungal biomass) for the removal of chromium. The Cr (VI) reduction was observed under all the culture conditions, like 90-99% occurred due to the fungal biomass (Sahin and Ozturk *et al.,* 2004). *Aspergillus* sp. and *A. flavus* were capable to reducing all the pH concentrations, utilizing all the temperatures, and reducing the chromium rate. The optimum pH is 2.0 and temperature at 30 °C for 150 mg/L of chromium. Similarly, the chromium metal reduction was concluded to have an optimum pH at 2 and pH of 5.5 for *Paecilomyces lilacinus* (Reuel *et al.,* 2013).

The present study reported that removal of Cr(VI) using fungal biomass (adsorbents) falls into two categories, active fungal biomass and inactive fungal biomass. The active fungal biomass when compared to inactive fungal biomass, has a high amount of Cr(VI) removal rate, whereas inactive fungal biomass was observed in all the optimization studies and column studies also included. The whole part of the study was planned into one potential strain and based on the secondary screening Cr(VI) removal rate, one potential was selected for further studies. The removal rate of active at S12 was 92.63% and that of inactive fungal biomass at 98.01%.

Xu *et al.,* (2022) revealed the optimum inoculum for Cr(VI) removal at 2%. The varying inoculum studied were 0.5, 1, 1.5 and 2% inoculum. The effect of varying

inoculums was studied at five different concentration of both active and inactive fungal biomass for Cr(VI) removal. The optimum inoculum at 1.5% in active fungal biomass and 1% in inactive fungal biomass with a high amount of Cr(VI) removal rate is calculated. Finally, the Cr(VI) removal rate in active fungal biomass about 84.67% and in inactive fungal biomass is 97.75%.

The effects of pH were studied. Cr(VI) reduction in pH 1-3 was better reduction noted for fungus and algae (Ramrakhiani *et al.,* 2011). Cabatingan (2001) revealed Cr(VI) reduction of optimum pH at 6.0, overall pH 2-6 were studied. Xu *et al.,* 2022, represent the optimum pH for Cr(VI) reduction, which occurs at a pH of 2.0 and pH 1-8 for this study. The active and inactive fungal biomass were utilized to reduce the Cr(VI) all the pH levels, with the optimum pH at 3. Because the optimum pH 3.0 is compared to other pH (pH – 3, 5, 7, 9, and 11), Cr (VI) removal is rapid condition. Finally, the Cr (VI) removal rate of optimum pH active fungal biomass 93.69% and that of inactive fungal biomass 96.80%. The optimum temperature is 35°C for both active and inactive fungal biomass Cr(VI), and all the temperatures are utilized when compared to the optimum temperature for fastidious Cr(VI) removal. The Cr(VI) removal rate was monitored for active fungal biomass at 93.60% and inactive fungal biomass at 99.80%

Finally, the column study states that, fastidious removal rate of Cr(VI) is active fungal biomass at 89.67% and inactive fungal biomass at 94.55%. Park *et al.,* 2007 and Xu *et al.,* 2022 state that, the mycelial pellets are white in color and have a diameter of about 3 mm. The surface area of the mycelial pellets could be entangled with 3-dimensional network and this is supposed to have its own surface area. The study also revealed the adsorption-coupled reduction mechanism for Cr(VI) removal. The SEM images showed the presence and absence of chromium (VI) cell accumulation mycelial pellets. EDX were conformed before and after treatment of chromium (VI), and it maybe Cr (VI) or Cr (III). The previous study reported Cr(VI) reduction of treated biomass in the carboxyl and amine group. The Cr(VI) reduction was monitored 1 hour and 24 hours. (Sanghia *et al.,* 2009). Dhal *et al.,* 2018 state that carboxylate, amine, amide and hydroxyl functional groups were conformed under the microfibrils of fungal cell wall. Shoaib *et al.,* 2013 reported that the fungal biomass observed amine and alcohol group for Cr(VI) reduction. The FTIR results, with and without chromium (VI) find out the amine, alcohol, alkene and carbon oxide compounds. These functional groups confirm the accumulation of spore-enriched mycelial pellets with chromium (VI).

In conclusion, the Chromium tolerant fungus *Aspergillus flavus (*S12) were studied into active and inactive fungal biomass (adsorbent) for Cr(VI) into Cr(III) in aqueous medium. The effects of inoculum, pH, and temperature on the lab scale column were investigated.

The optimization studies revealed the Cr(VI) removal rate, and Cr(III) was also noted. The active biomass represents 89.67% and inactive biomass showed the 94.55% of the Cr(VI) removal rate in this study. Finally, the before and after treatment of Cr(VI) were find out using SEM-EDX and FTIR analysis for Cr(VI) removal studies.

The mechanism of whole study Cr(VI) removal was studied in fungal biomass under both active and inactive fungal biomass conditions. The biomaterial of active and inactive fungal biomass was used as adsorbents for Cr(VI) removal studies in aqueous medium. The fungal biomass was used for waste water treatment and was a very effective method for Cr(VI) removal.

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Author Contributions

V. Suruthi: Investigation, formal analysis, writing—original draft. S. Rajakumar: Validation, methodology, writing—reviewing. P. M. Ayyasamy:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Abdulmalik, A.F., Yakasai, H.M., Usman. S., Muhammad, J.B., Jagaba, A.H., Ibrahim, S., Babandi, A and Shukor, M.Y. 2023. Characterization and invitro toxicity assay of bio-reduced hexavalent chromium by *Acinetobacter* sp. isolated from tannery effluent. Case Studies in Chemical and Environmental Engineering. 2666-0164. https://doi.org/10.1016/j.cscee.2023.100459

Ahmad, L.M., Bashir, A., Qureashi, A and Hussain, A.P. 2019. Detection and removal of heavy metal ions: a review. Environmental Chemistry Letters. 17:1495-1521. https://doi.org/10.1007/s10311-019-00891-z

Ali, H., Khan, E and Ilahi, I. 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. Journal of Chemistry. 2019, 6730305. https://doi.org/10.1155/2019/6730305

Ayangbenro, A.S and Babalola, O.O. 2018. Environments: A New Strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents. Int. J. Environ. Res. Public Health. 14, 94. http://doi.org/10.1016/j.ijerph14010094

Ayyasamy, P.M and Lee, S. 2009. Redox transformation and biogeochemical interaction of heavy metals in Korean soil using different treatment columns in the presence of *Shewanella* sp. Chemosphere. 77: 501-509. https://doi.org/10.1016/j.chemosphere.2009.07.052.

Beukes, J.P., Pienaar, J.J., Lachmann, G and Giesekke, E.W, 1999. The reduction of hexavalent chromium by sulphite in wastewater. 25 (3)0378-4738.

Cabatingan, L.K., Ramelito, C., Agapay., Rakels, J.L.L., Ottens, M and wielen, L.A.M.V.D. 2001. Potential of Biosorption for the Recovery of Chromate in Industrial Wastewaters. Industrial and Engineering Chemistry Research. 40 (10) 2302-2309. https://doi.org/10.1021/ie0008575.

Carolin, C.F., Kumar, P.S., Saravanan, A., Joshiba, G.J and Naushad, M. 2017. Efficient techniques for the removal of toxic heavy metals from aquatic environment: a review. J.Environ. Chem. Eng. 5, 2782-2799. https://doi.org/10.1016/j.jece.2017.05.029

Dhal, B., Abhilash and Dhar, B.P. 2018. Mechanism elucidation and adsorbent characterization for removal of Cr(VI) by native fungal adsorbent. Sustainable Environment Research. 28, 289e297. https://doi.org/10.1016/j.serj.2018.05.002.

Fatima, A.A., Muhammad, H.Y., Usman, S., Bashir, J.M., Hussaini, A.J., Ibrahim, S., Babandi, A and Yunus, M.S. 2023. Characterization and invitro toxicity assay of bio-reduced hexavalent chromium by *Acinetobacter* sp. isolated from tannery effluent. Case Studies in Chemical Environmental Engineering. 8, 100459. https://doi.org/10.1016/j.cscee.2023.100459

Gautam, A., Kushwaha, A and Rani, R. 2022. Reduction of Hexavalent Chromium [Cr(VI)] by Heavy Metal Tolerant Bacterium *Alkalihalobacillus clausii* and its Toxicity Assessment through Flow Cytometry. Current Microbiology. 79:33. https://doi.org/10.1007/s00284-021-02734-z.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B and Beeregowda, K.N. Toxicity, mechanism and health effects of some heavy metals. Interdiscip. Toxico. 7, 60-72. https://doi.org/10.2478/intox-2014-0009.

Kahlon, S.K., Sharma, G., Julka, J.M., Kumar, A., Sharma, S and Stadler, F.J. 2018. Impact of heavy metals and nanoparticles on aquatic biota. Environmental Chemistry Letters. 16:919-946. DOI, journal name. https://doi.org/10.1007/s10311-018-0737-4

Kim, J.J., Kim, Y.S and Kumar, V. Heavy metal toxicity: an update of chelating therapeutic strategies. J. Trace Elem. Med. Biol. 54, 226-231. https://doi.org/10.1016/j.jtemb.2019.05.003.

Lebedev, A., Sinikova, N., Nikolaeva, S., Poliakova, O., Khrushcheva, M and Pozdnyakov, S. 2003. Metals and organic pollutants in snow surrounding an iron factory. Environmental chemistry Letters. 1:107-112. https://doi.org/10.1007/s10311-002-0004-5.

Mohsenzadeh, F and Shahrokhi, F. 2014. Biological removing of cadmium from contaminated media by fungal biomass of *Trichoderma* species. Journal of enivrionmental health science and

engineering. 12:102. https://doi.org/10.1186/2052-336X-12-102

Park, D., Lim, S.R., Yun, Y.S and Park, J.M. 2007. Reliable evidences that the removal mechanism of hexavalent chromium by natural biomaterials is adsorption-coupled reduction. Chemosphere. 70 (2) 298-305. https://doi.org/10.1016/j.chemosphere.2007.06.007.

Park, D., Yun, Y.S., Jo, J.H and Park, J.M. 2005. Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*. Water Res. 39 (4) 533-540. https://doi.org/10.1016/j.waters.2004.11.002.

Puyen, Z. M., Villagrasa, E., Maldonado, J., Diestra, E., Esteve, I., & Solé, A. (2012). Biosorption of chromium by Acinetobacter sp. and Arthrobacter sp. bacterial consortium. Journal of Hazardous Materials, 213–214, 242–248. https://doi.org/10.1016/j.jhazmat.2012.01.061

Qian, J., Zhou, J., Wang, L., Wei, L., Li, Q., Wang, D and Wang, Q. 2017. Direct Cr(VI) bio-reduction with organics as electron donor by anaerobic sludge. Chemical Engineering Journal. 1385-8947. https://doi.org/10.1016/j.cej.2016.10.077.

Rafi, S., Shoaib, A., Arshad, Z.A., Batool, N.R., Nafisal and Shafi, M. 2017. Chromium tolerance, oxidative stress response, morphological characteristics, and FTIR studies of phytopathogenic fungus *Sclerotium rolfsii*. Folia Microbiol. 62:207-219. https://doi.org/10.1007/s12223-016-0489-0.

Ramrakhiani, L., Majumder, R and Khowala, S. 2011. Removal of hexavalent chromium by heat inactivated fungal biomass of Termitomyces clypeatus: Surface characterization and mechanisms of biosorption. Chemical Engineering journal. 171 (3) 1060-1068. https://doi.org/10.1016/j.cej.2011.05.002

Ren, B., Zhang, Q., Zhang, X., Luyang, Z.Z and Li, H. 2018. Biosorption of Cr(VI) from aqueous solution using dormant spores of *Aspergillus* niger. Royal Society of Chemistry. 8, 38157-38165. https://doi.org/10.1039/c8ra07084a.

Reuel, M.B., Rodrigo, P.F., Cordero., Gershon, S., Bautista and Gina, R.D. 2013.Reduction of hexavalent chromium using fungi and bacteria isolated from contaminated soil and water samples. Chemistry and Ecology.

Sahin, Y and Qzturk, A. 2004. Biosrption of chromium(VI) ion from aqueous solution by the bacterium *Bacillus thuringensis*. Process Biochemistry. 40; 1895-1901. https://doi.org/10.1016/procbio.2004.07.002.

Sanghia, R., Sankararamakrishnana, N., Bakul and Dave. 2009. Fungal bioremediation of chromates: Conformational changes of bomass during sequestration, binding, and reduction of hexavalent chromium. Journal of Hazardous Materials. 169, 1074-1080. https://doi.org/10.1016/j.jhazmat.2009.04.05

Sheng, D.X., Long, Y.L., Qiang, D.L., Jie, C.G and Jing, S.Y. 2017. Halostable catalytic properties of exoglucanase from a marine *Asoergillus niger* and secondary structure change caused by high salinities. Process biochemistry. 1359-5113. https://doi.org/10.1016/j.procbio.2017.02.005.

Shoaib, A., Aslam, N., Makshoof, M.A., Akhtar, S., Nafisa., Khurshid, S. 2013. Removal of Cr(III) through Bread Mold Fungus. Polish Journal of Environmental Studies. 22, 4, 1171-1176.

Siddiquee, S., Rovina, K., Azad, S.A., Naher, L., Suryavani, S and Chaikaew, P. 2015. Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: A review. Journal of Microbial and Biochemical Technology. 7(6), 384-395. https://doi.org/10.4172/1948-5948.1000243.

Silvia, A.T., Aranguiz, C., Alejandre, M.S.N., Alejandra, M.P and Isabel, M.F. 2023. Fungal species originating from chromium contaminated soil for ecofriendly and biotechnological processes. Applied soil biology. 0929-1393. https://doi.org/10.1016/j.apsoil.2023.105231

Singh, S.A and Goyal, D. 2010. Removal of Cr(VI) from aqueous solution by fungal biomass. Eng. Life. Sci. 5, 480-485. https://doi.org/10.1002/elsc.200900111.

Srivastava, S., Ahmad, A.H and Thakur, I.S. 2008. Removal of chromium and pentachlorophenola from tannery effluents. Brioresource technology. 98, 1128-1132. http://dx/doi.org/10.1016/j.biortech.2006.04.011.

Su, C., Jiang, L.Q and Zhang, W.J. 2014. A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques. Environmental Skeptics and Critics. 3(2), 24.

Tarekegn, M.M., Salilih, F.Z and Ishetu, A.I. 2020. Microbes used as a tool for bioremediation of heavy metal from the environment. Cogent Food and Agriculture. 6: 1783174. https://doi.org/1.1080/23311932.2020.1783174.

Wang, H., Wu, Z.K., Chen, B.B., He, M and Hu, B. 2015. Chip-based array magnetic solid phase micro extraction on-line coupled with inductively coupled plasma mass spectrometry for the determination of trace heavy metals in cells. Analyst. 140:5619-5626. https://doi.org/10.1039/C5AN00736D

Xu, S., Zhang, Q., Bai, D., Cai, L., Lu, T and Yao, S. 2022. Removal process and mechanism of hexavalent chromium byadsorption-coupled reductionwith marine-derived *Aspergillus niger* mycelial pellets. Chinese Journal of Chemical Engineering. 1004-9541. https://doi.org/10.1016.j.cjche02021.10.002.

Xu, S., Zhang, Q., Bai, D., Cai, L., Lu, T and Yao. 2022. Removal process and mechanism of hexavalent chromium by adsorption-coupled reduction with marine-derived *Aspergillus niger*. 49, 198-204, 1004-9541. https://doi.org/10.1016/j.cjche.2021.10.002.

Xue, D.S., Chen, Y.R., Ren and Yao, S.J. 2012. Enhancing the activity and thermostability of thermostable β-glucosidase from a marine *Aspergillus niger* at high salinity. Process Biochem. 47 (4) 606-611. https://doi.org/10.1016/j.procbio.2011.12.021

Ying, H.C., Xin, Y.G and Jing, G.Y. 2013.A novel two-species whole-cell immobilization system composed of marine-derived fungi and its application in wastewater treatment. Journal of chemical technology and Biotechnology. 89 (11), 1733-140. https://doi.org/10.1002/jctb.4253.

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