

**ORIGINAL RESEARCH PAPER**

## **Biosorption of hexavalent chromium in a tannery industry wastewater using fungi species**

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**ABSTRACT:** The isolated fungi species of different kinds from chromium contaminated soil sites located in Nagalkeni, Chennai were used for reducing chromium(VI) in a tannery industry wastewater of Nagalkeni, Chennai. The experiments were conducted to know biosorption potential of isolated fungi species for removing chromium(VI) in a tannery industry wastewater against the different pH, fungi biomass and chromium(VI) concentration (dilution ratio). The results of this study indicated that the order of maximum removal of chromium(VI) by an isolated fungi species at an optimum pH of 3, fungi biomass of 4g and an initial chromium(VI) concentration of 18.125 mg/L (dilution ratio 4) is *A. niger* > *A. flavus* > *A. fumigatus* > *A. nidulans* > *A. heteromorphus* > *A. foetidus* > *A. viridinutans*. This study found that the maximum removal of chromium(VI) was achieved by *Aspergillus niger* (96.3 %) than other fungi species at chromium(VI) concentration of 18.125 mg/L in a tannery industry wastewater. The chromium removal from tannery industry wastewater was validated by checking chromium removal in an aqueous solution and by checking the removal efficiency of other parameters in a tannery industry wastewater using same isolated *A. niger*. Biosorption model was proposed to simulate the experimental condition for removing chromium(VI) in a tannery industry wastewater by all isolated fungi species. The  $R^2$  and  $\chi^2$  values of the proposed model predicted that the proposed biosorption model is very much useful for predicting the trend of reduction potential of chromium(VI) in a tannery industry wastewater by all isolated fungi species. This study suggested that one could select the type of fungi species, ion concentration level, selection of treatment period, quantity of biomass to be used, and pH level of the medium, to achieve the highest reduction of any toxic metals from any contaminated water, wastewater and soil environment.

**KEYWORDS:** *Biosorption Model; Chromium (VI) tolerant; Fungi species; Minimum incubatory concentration; Tannery Industry; Tolerance index; Wastewater*

### **INTRODUCTION**

Contamination by toxic metals is a serious environmental issue of global concern. In developing countries, toxic metals containing effluent are discharging continuously from several thousands of small and large-scale industries such as metallurgy,

battery manufacturing, leather tanning, chemical manufacturing, mine drainage, electroplating industries, solid waste dumping yard drainage (leachate) etc., directly or indirectly reaching to natural water resources, posing a major threat to the environment (Fukuda *et al.*, 2008a,b; Sivakumar 2011, 2013a). The presence of toxic metals like Cu, Zn, Ni, Pb, Cd, and Cr from the drinking water sources poses a great threat to public health if exceeds the standard limit (Bureau of Indian Standards Drinking water

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specification, 2012). Among various toxic metals, trivalent and hexavalent forms of chromium is contaminated the environment, however, Chromium (VI) is of particular concern due to its greater toxicity. Human is facing the problems like epigastric pain, nausea, vomiting, gastric system, severe diarrhea, skin irritation, respiratory tract and lung carcinoma when chromium polluted water consumed. Due to this reason, chromium in contaminated water has to be removed.

Removal of Cr(VI) ions from industrial wastewater is achieved by the application of several conventional processes such as reduction, chemical precipitation, adsorption (Sivakumar 2013b; Sivakumar *et al.*, 2014c), sedimentation, electro-dialysis (Sivakumar *et al.*, 2014e), ion exchange, biological operations (Sivakumar *et al.*, 2014d), cementation, filtration, coagulation, flocculation, membrane processes, and solvent extraction. These methods require more usage of chemical substances and energy and they generated hazardous by-products. Naturally occurring microorganisms provide viable option for detoxifying Cr(VI), such a way it protect the environment.

Bacteria, fungi, yeast and algae are available in nature abundantly, which are potential alternative to conventional methods used to detoxify liquid wastes (Shankar *et al.*, 2014; Sivakumar *et al.*, 2014a,b). Among the available various microorganisms, fungi can tolerate and survive in the presence toxic metals from the contaminated soil. Survive of fungi in toxic metal contaminated sites is due the presence of cell wall material within that shows excellent metal-binding properties (Shazia *et al.*, 2013a,b). In addition, fungi species are known to detoxify toxic metals by several mechanisms, including extra and intra cellular precipitation, valence transformation and active uptake (Mala *et al.*, 2006; Turnau *et al.*, 2006), and biosorption to cell wall pigments (Islam *et al.*, 2008; Islam, *et al.*, 2011). Similarly, it can adapt and grow under high metal concentrations and in various extreme conditions of temperature, nutrient availability and pH (Anand *et al.*, 2006). However, phosphate, proteins, and nitrogen-containing ligands on protein, chitin and chitosan are also influencing the toxic metal uptake efficiency of fungi species (Bai, *et al.*, 2012; Chen *et al.*, 2012).

The geniuses *Aspergillus* are survived in different climatic conditions of worldwide. *Aspergillus* species

commonly grow as molds on the surface of a substrate and *Aspergillus* species are used for waste management and biotransformations (Gupta, *et al.*, 2012). Species of different kinds of *Aspergillus* have been reported as efficient removal of Cr(VI) (Shazia *et al.*, 2012) and *Aspergillus* has also been used to remove cadmium and copper ions from aqueous solutions (Kapoor and Cullimore 1999). The most common fungal strains *Fusarium*, *Aspergillus* and *Penicillium* species were isolated from irrigated soil with municipal and or industrial wastewater and the same were tested for tolerance against the toxic metals Zn, Pb, Ni and Cd in wastewater (Shazia, *et al.*, 2009). The fungal species such as *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus* were also isolated from sewage contaminated agricultural soil and industrial wastewater and the same is used for reduction of Cr and Pb contaminated water (Shazia *et al.*, 2013). Isolated seven *Aspergillus* fungi species and three other fungi species such as *Curvularia*, *Acrimonium* and *Pithyium* from soils irrigated with untreated municipal/industrial effluent were used successfully for bioremediation of Cd, Ni and Cu in a contaminated soil and wastewaters (Akhtar *et al.*, 2013).

The two filamentous fungi of *Rhizopus* and *Aspergillus* species from metal contaminated agricultural soil were used for biosorption of Cd and Cr (Zafar *et al.*, 2007). *Rhizopus arrhizus* (Aksu and Balibek 2007), *Penicilium spinulum* (Fukuda *et al.*, 2008b) and *Aspergillus niger* (Acevedo-Aguilar *et al.*, 2008; Fukuda *et al.*, 2008b) have been extensively studied against Cr(VI) tolerance potential and the results of studies showed that the removal mechanism depends on type of species used. A *Paecilomyces* fungal strain exhibits more absorption, high resistance and high reduction potentials of Cr(VI) than other fungi species (Fukuda *et al.*, 2008b).

Thus, this study focused to isolate different fungi species from the chromium contaminated soil, where tannery industrial wastewater was discharged. The study was conducted with the specific aims of establishing the removal potential of isolated fungi species for removing Cr(VI) in a tannery industry wastewater at Nagalkeni, Kanchipuram, Tamil Nadu. The present study also provided an idea for researchers to carryout further research on bioremediation using various fungi species for reducing Cr(VI) from contaminated water and soil environment. This study was conducted in the month of March 2015.

## MATERIALS AND METHODS

### Study area

Nagalkeni, situated in Kanchipuram District, Tamil Nadu with 12.96 Latitude and 80.14 Longitude (Fig. 1) was identified for this study. The porous media (the soil) and groundwater in Nagalkeni was contaminated due to discharge on land by untreated and partially treated wastewater of tannery industry. Chromium available in tannery industry wastewater exists in the form of Cr(III) and Cr(VI). Cr(III) is only toxic at high concentrations, whereas hexavalent Cr(VI), is considered more toxic to all living beings and the environment when concentration of Cr(VI) exceeds the tolerance limit of 0.05 mg/L (BIS drinking water specification - IS 10500:2004). The contaminated soil (porous media) in Nagalkeni was used for isolating different fungi species, which in turn used for removing Cr(VI) from tannery industry wastewater of Nagalkeni.

### Soil sample collection

In order to isolate the various fungi species, soil samples were collected from tannery industry wastewater contaminated site, located in Nagalkeni (Fig. 1). When tannery industry wastewater is disposed onto agricultural fields, chromium enters through the porous of soil environment and gets fixed within soil stratum, which in turn accumulates into the plants when plants are growing on land and finally it reaches to human being when human being consumed the plants in various forms. The persistent nature of chromium in the soil environment for a long period, the soil environment lost its characteristics (Munees and Mulugeta 2013).

The collected tannery industry contaminated soil samples were spreading as a thin layer in an air drying tray after the clods are breaking by fingers or a hammer. The soil samples were then dried with a temperature of

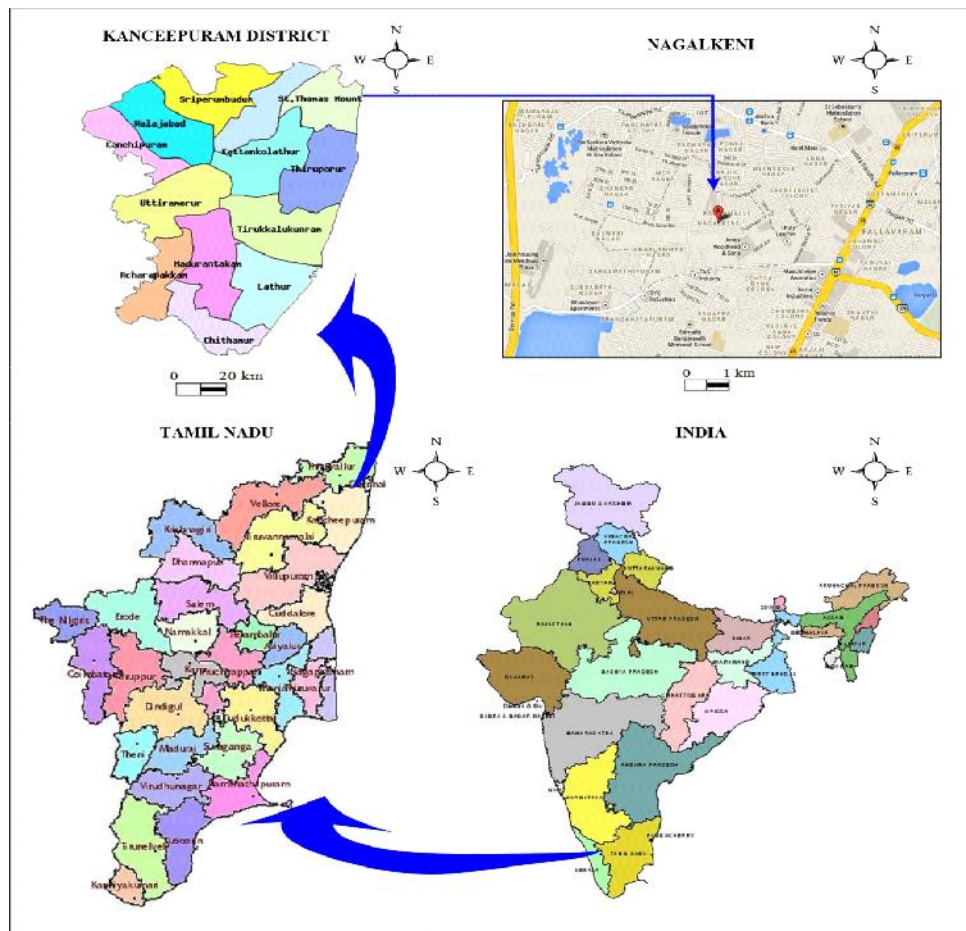


Fig. 1: Study area of Nagalkeni

not exceeding 30 °C by air blowing. The air blow drying process was extended until the soil samples get dry. After air drying, and after removing stones and debris, the soil samples were grinded with mortar and pestle, such a way, the soil grain size was maintained as 2 mm after passing through 2 mm mesh sieve.

Most of the Cr(VI) tolerant fungi species are surviving only in the top layer of soil stratum, where, the fungi species are getting oxygen for their survival. The available quantity of oxygen is getting depleted as depth increases from the soil stratum and hence, less numbers and quantity of fungi species are surviving in a greater depth. So, for this study, the soil samples were collected at the depth of not exceeding 5 cm from the surface soil stratum for identifying and isolating the Cr(VI) tolerant fungi species (Shazia *et al.*, 2009, 2013b). The digging tools and spatula were used for collecting the soil samples. Soil samples were collected at 5 places around the contaminated site randomly and composite soil sample was prepared by mixing of soil samples from 5 sites and it was taken to an environmental engineering laboratory for analyzing the chromium content.

The composite soil sample 1 g was taken in the 50 mL conical flask, added 10 mL of HNO<sub>3</sub>:HClO<sub>4</sub> (1:2) solution (50 mL) and heated at a temperature of 95 °C without boiling for half an hour. The heated solution was filtered through Whatman filter paper and volume was made to 50 mL by adding deionized water (Shazia *et al.*, 2009, 2012, 2013a, 2013b). Triplicates of composite soil samples were digested and analyzed for Cr(VI) ion (APPA, AWWA, and WEF, 2005). The blank sample contained 10 mL of HNO<sub>3</sub>:HClO<sub>4</sub> (1:2) solution and heated at a temperature of 95 °C without boiling for half an hour and volume was made 50 mL by adding deionized water was used for checking the quality assurance of samples. The solution of soil sample was stored at 4 °C to ensure minimal biological activity. At storage condition, tannery industry wastewater contaminated soil sample was maintained with the pH of 8, is used not to oxidize Cr(III) into Cr(VI). The isolation of fungi species was carried out within 24 h of contaminated soil sample collection for further investigation.

#### Wastewater sample collection

Totally, 5 wastewater samples were collected without the presence of air bubbles using cleaned airtight plastic bottles at Nagalkeni, Pallavaram, Chennai, where

clusters of tannery industries are available. In the study area, 5 sampling points were selected randomly such that sample 1 was collected from wastewater disposal point, samples 2 and 4 were collected away and far away respectively on the right side of the wastewater disposal point. Similarly, samples 3 and 5 were collected away and far away respectively on the left side of the wastewater disposal point. The collected 5 samples were mixed together to make a composite sample and it was stored at 4 °C in an environmental engineering laboratory for determining the Cr(VI). The pH in a tannery industry wastewater at the time collection is 4.3 (Table 1), at pH 4.3, the Cr(VI) is in the form of Cr<sub>2</sub>O<sub>7</sub><sup>-2</sup>, which are unstable in nature. Hence, as similar to storage of soil solution, pH of the tannery industry wastewater was maintained at 8 for the storage and is used not to oxidize Cr(III) into Cr(VI). Since, at pH 8, Cr(III) is very stable and Cr(III) exists primarily as a cation in tannery industry wastewater, i.e., in the form of hydroxide precipitate. Furthermore, when the tannery industry wastewater approaching neutral pH, Cr(III) will be formed a hydroxo-Cr(III) species and approaching acidic pH, Cr(III) is oxidized to Cr(VI) and in the form of CrO<sub>4</sub><sup>-2</sup> and Cr<sub>2</sub>O<sub>7</sub><sup>-2</sup>.

#### Determination of Cr(VI) in wastewater

Hexavalent Chromium was quantified with the help of UV spectrophotometric method employing 1, 5-diphenylcarbazide as complexing agent (Shugaba *et al.*, 2012). One milliliter (1 mL) of 0.2 % w/v of 1, 5-diphenylcarbazide solution (prepared in 95 % ethanol and 1 mL of 1/5 H<sub>2</sub>SO<sub>4</sub>) was added to 1 mL of the sample solution (APPA, AWWA, and WEF, 2005). The absorbance of Cr(VI) was determined by using Elico-UV spectrophotometer, SL 150 adjusted at  $\lambda_{max}$  (Fig. 2). From Fig. 2, it was found that absorbance increased as wavelength increased, furthermore, for further increased wavelength, the absorbance got decreased. The point of absorbance decreased is called point of deflection and the maximum wavelength is obtained

Table 1: Characteristics of tannery industry wastewater

Sl No.	Characteristics	Values
1	pH	4.2
2	Cr(VI)	290 mg/L
3	TDS	11890 mg/L
4	TS	15650 mg/L
5	COD	7865 mg/L
6	BOD	5325 mg/L
7	Sulphate	792 mg/L

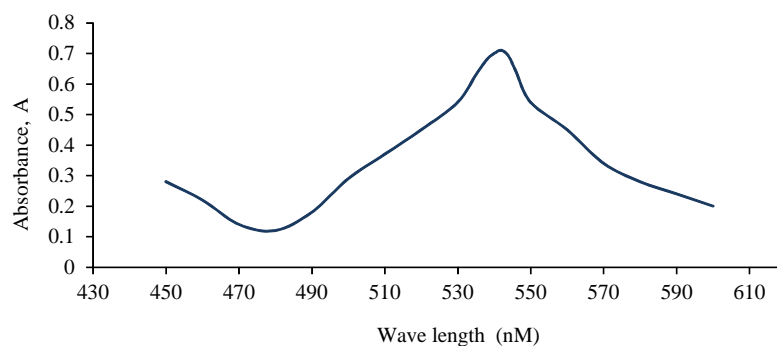


Fig. 2: Absorbance curve for standard Cr(VI) ion solution

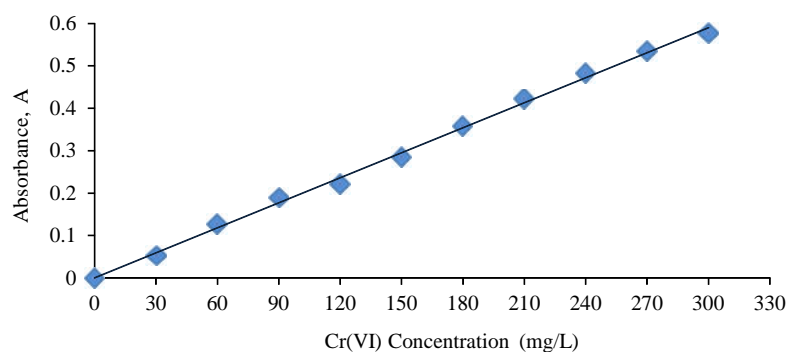


Fig. 3: Calibration curve for standard Cr(VI) ion solution for the maximum absorbance wavelength of 542 nM

corresponding to the point of deflection. Thus, the maximum wavelength found for the Cr(VI) absorbance is 542 nM (Fig. 2).

The Cr(VI) concentration in a tannery industry wastewater was extrapolated from a standard curve prepared from standard solutions of potassium dichromate. Fig. 3 indicates a calibration curve for standard Cr(VI) ion solution for the maximum absorbance wavelength of 542 nM and it was observed that as a dilution factor increases absorbance increases linearly with  $R^2$  value of 0.9975. Thus, Fig. 3 is used for determining Cr(VI) concentration in a tannery industry wastewater of Nagalkeni, before and after treating with isolated fungi species.

Furthermore, various physico-chemical parameters such as pH, total dissolved solids (TDS), total solids (TS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), and sulphate ( $SO_4^{2-}$ ) in a homogenized tannery wastewater were also analyzed before and after treating with isolated fungi species (APPA, AWWA, and WEF, 2005), which is turn used for validating the experimental results of Cr(VI) removal.

The physico-chemical characteristics of Cr(VI) and other parameters in a tannery industry wastewater are given in Table 1.

#### *Sterilization of apparatus*

The petri plates, media bottles, deionized water, McCartney bottles and syringes were sterilized in an autoclave for 60 min at 120 °C. After autoclaving, all sterilized materials were dried in an oven at 100 °C.

#### *Media preparation*

Potato Dextrose Agar (PDA) medium was used to isolate the various fungi species. For the preparation of PDA, 200 g of potatoes was peeled, sliced and boiled, and then sieved through a clean muslin cloth to get a broth in which 10 g of agar and 10 g of dextrose sugar were added. The media was then autoclaved for 30 min at 120 °C.

#### *Isolation of fungi*

Fungi species were isolated on PDA by the soil dilution method. Poured the media in Petri-dishes and

allowed to solidify for 48 h. To suppress the bacterial growth, 25 mg/L of streptomycin was added to the medium (Shazia *et al.*, 2013). After solidification, the plates were filled with diluted soil solution (different proportions). The plates were incubated at 28 °C for 72 h. The prominent colonies were picked after incubation periods, and inoculated individually in other PDA plates for further purification.

#### Identification of fungi

After an incubation period of 72 h, the distinct colonies were counted. The fungal cultures were identified on the basis of macroscopic (colonial morphology, texture, shape, colour, diameter and appearance of the colony) and microscopic (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia, and presence of sterile mycelium) characteristics (Shazia *et al.*, 2012; Zafar *et al.*, 2007). The morphological characteristics of isolated fungi species from tannery industry wastewater contaminated soil is given in Table 2.

#### Biosorption study

Batch biosorption experiments were conducted by glass bottles containing tannery industry wastewater with various pH (1, 2, 3, 4 and 5), fungus biomass (1, 2, 3, 4 and 5 g), and dilution ratios (0, 1, 2, 3 and 4) against different incubation time (0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 days). At the end of selected incubation period, a

tannery industry wastewater treated with isolated fungi species was taken from the incubator and filtered through Whatman 1 filter paper. The filtrate's absorbance was determined by using Elico-UV spectrophotometer, SL 150 adjusted at  $\lambda_{max}$  (Fig. 2). By referring to the calibration curve of the absorbance (Fig. 3), obtained Cr(VI) concentration in a treated tannery industry wastewater for different process parameters by an isolated fungi species. The Cr(VI) removal percentage in a tannery industry wastewater by an isolated fungi species was calculated by using the Eq. 1.

$$\text{Percentage removal} = \frac{(C_1 - C_2)}{C_1} \times 100 \quad (1)$$

in which  $C_1$  and  $C_2$  are the concentration of the Cr(VI) in a tannery industry wastewater before and after the treatment with isolated fungi species respectively. Furthermore, by referring to the calibration curve (Fig. 3), Cr(VI) was measured before and after treating the isolated fungi from chromium contaminated soil with tannery industry wastewater. The values presented in this study are the average values from triplicate samples.

## RESULTS AND DISCUSSION

In this study, Cr(VI) resistant fungi species were isolated from tannery industry wastewater contaminated sites, which are labeled as F1, F2, F3, F4, F5, F6 and F7 respectively for *A. niger*, *A. flavus*, *A.*

Table 2: Morphological characteristics of isolated fungi species from tannery industry wastewater contaminated soil

Fungi species	Appearance of colonies
<i>Aspergillus niger</i> ( <i>A. niger</i> )	The surface is velvety texture in nature, woolly initially white, quickly becoming black with conidial production. Yellow reverse of petriplate, zonation is heavily furrowed on the reverse and growth may produce radial fissures from the center.
<i>Aspergillus flavus</i> ( <i>A. flavus</i> )	Initially yellowish colour and it turn to green when aging. The conidial reverse is yellow colour. The colonies grow rapidly. The conidial heads are lime colour.
<i>Aspergillus fumigatus</i> ( <i>A. fumigatus</i> )	The colonies are dark blue green to gray green on the surface, reverse pale or yellow. The rapid growth of colonies. The surface is powdery texture. Conidial heads are typically columnar and uniseriate.
<i>Aspergillus nidulans</i> ( <i>A. nidulans</i> )	The texture of species is lanose, at the beginning, the colony surface colour is white, then ochre, center of the colony is lightly raised. The reverse is dark purple like eggplant. Colourless exudates on the surface. Conidial heads are short and columnar.
<i>Aspergillus heteromorphus</i> ( <i>A. heteromorphus</i> )	The boundary of the colony is greenish black. Conidial heads at margin are small, thin, smooth, richly sporulated and yellowish in nature whereas in the center more sporulated. The reverse is colourless, no distinct odour, and no exudate.
<i>Aspergillus foetidus</i> ( <i>A. foetidus</i> )	Texture is lanose, white colour in boundary and yellowish in nature at center. Sporulation is more at colony boundary and at the center. It contains blackish brown conidial heads. The reverse is bright yellow. There is zonation and the mycelium is golden yellow.
<i>Aspergillus viridinutans</i> ( <i>A. viridinutans</i> )	The colony colour is white at first and then turns to green at the center. The reverse is light brown. Exudates are small and colourless with no zonation. The surface is light green-white.

Table 3: The isolated fungi species from contaminated soil samples

Fungi symbol	Isolated fungi	Sample codes
F1	<i>Aspergillus niger</i>	S1, S2, S3, S5, S6
F2	<i>Aspergillus fumigatus</i>	S8, S11, S12
F3	<i>Aspergillus nidulans</i>	S13, S14
F4	<i>Aspergillus foetidus</i>	S15, S16
F5	<i>Aspergillus flavus</i>	S4, S7, S9, S10
F6	<i>Aspergillus heteromorphus</i>	S17, S18
F7	<i>Aspergillus viridinutans</i>	S19, S20

*fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans* (Table 3). All seven isolated fungi species were obtained from soil dilution method and are labeled as S1 to S20 (Table 3). The sample codes from S1 to S20 indicate the dilution ratio starts from 0 to 20 respectively. The isolated fungi species (from S1 to S20) from tannery industry wastewater contaminated soil is used to remove Cr(VI) ions from tannery industry wastewater of Nagalkeni. The experiments were conducted against the effect of pH, fungi biomass and different Cr(VI) concentration (different dilution ratio) to know the effectiveness of isolated fungi species (from S1 to S20) for removing Cr(VI) ion from a tannery industry wastewater. The results of this study are presented based on the average value of three replicates.

### Effect of pH

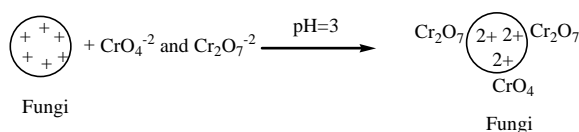
Table 4 represents the percentage removal of Cr(VI) by 1.0 g of each fungus biomass (from S1 to S20), an initial Cr(VI) concentration of 290 mg/L (dilution ratio 1 part of tannery industry wastewater : 0 parts of deionized water) and for the incubation period of 7 days (Since, day 7 is the optimum contact time found from the study, the results obtained on day 7 were presented and results obtained for the day 1, 2, 3, 4, 5, 6, 8 and 9 were not presented in this study) against various pH of 1.0, 2.0, 3.0, 4.0 and 5.0. At the time of experiment, the pH in a tannery industry wastewater is adjusted by adding an ammonium nitrate solution buffered between pH 1 and 5 to match the pH of the tannery industry wastewater. It may be observed from Table 4, the uptake of Cr(VI) is maximum at pH of 3 than at pH 1, 2, 4, and 5. The results revealed that the percentage removal of Cr(VI) is low at the pH 1 and 2, because, at pH 1 and 2, the quantity of fungi biomass is not sufficient to take up the presence of more quantity of Cr(VI) in a tannery industry wastewater. Similarly, for the pH 4 and 5, the availability of Cr(VI) ion in a tannery industry wastewater is less, since Cr(VI) ion is started to form Cr(III) ion, leading to lower specific uptake for pH of 4 and 5.

The another important observation made in this study is, when pH is 1 to below 7, chromium is present in +6 (as  $\text{Cr}_2\text{O}_7^{-2}$  and  $\text{CrO}_4^{-2}$ ) and +3 (as aquated  $\text{Cr}^{+3}$ )

Table 4: Effect of pH in removal percentage of Cr(VI) in tannery industry wastewater by isolated fungi from tannery industry wastewater contaminated soil against an optimum incubation time of 7 days, fungus biomass of 1.0 g, and an initial Cr(VI) concentration of 290 mg/L

Samples Codes	Fungi Species	Percentage removal of Cr(VI)				
		pH 1	pH 2	pH 3	pH 4	pH 5
S1	<i>A. niger</i>	79.5	85.6	92.5	82.4	75.4
S2	<i>A. niger</i>	77.7	83.6	90.4	80.5	73.6
S3	<i>A. niger</i>	74.9	80.6	87.1	77.6	71.0
S4	<i>A. flavus</i>	76.6	83.1	89.8	78.8	72.1
S5	<i>A. niger</i>	72.8	78.9	85.3	74.9	68.5
S6	<i>A. niger</i>	70.1	75.9	82.1	72.1	65.9
S7	<i>A. flavus</i>	74.0	80.2	86.7	76.1	69.6
S8	<i>A. fumigatus</i>	74.1	79.8	85.4	75.8	69.5
S9	<i>A. flavus</i>	71.8	78.0	83.4	72.9	66.9
S10	<i>A. flavus</i>	69.4	75.4	80.6	70.5	64.6
S11	<i>A. fumigatus</i>	71.0	77.0	82.4	72.1	66.1
S12	<i>A. fumigatus</i>	68.7	74.6	79.8	69.8	64.0
S13	<i>A. nidulans</i>	70.3	76.3	81.6	71.4	65.4
S14	<i>A. nidulans</i>	67.0	72.7	77.8	68.0	62.4
S15	<i>A. foetidus</i>	64.4	68.6	72.6	65.8	60.6
S16	<i>A. foetidus</i>	61.0	65.0	68.8	62.4	57.4
S17	<i>A. heteromorphus</i>	67.6	72.1	76.3	69.2	63.6
S18	<i>A. heteromorphus</i>	64.2	68.4	72.4	65.7	60.4
S19	<i>A. viridinutans</i>	60.2	64.1	67.8	61.7	56.8
S20	<i>A. viridinutans</i>	57.1	60.8	64.3	58.5	53.8

oxidation states and if  $\text{pH} > 7$ , chromium is in +3 (as  $\text{Cr}(\text{OH})_3$ ) oxidation state.  $\text{Cr}_2\text{O}_7^{-2}$  and  $\text{CrO}_4^{-2}$  are strong oxidizing agents. At the optimum pH of 3, chromium is present in +6 oxidation state as  $\text{CrO}_4^{-2}$  and  $\text{Cr}_2\text{O}_7^{-2}$ . The fungi with positive charges on them are adsorbed  $\text{CrO}_4^{-2}$  and  $\text{Cr}_2\text{O}_7^{-2}$ . At  $\text{pH} < 3$ , the fungi is not adsorbed  $\text{CrO}_4^{-2}$  and  $\text{Cr}_2\text{O}_7^{-2}$  because, both act as very strong anion and are not completely removed by the available number of positive charges on fungi species.



The Cr(VI) removal of all isolated fungi species has shown similar trends and an optimum pH found in this study for the maximum removal of Cr(VI) in a tannery industry wastewater is 3 (Table 4). The removal percentage of Cr(VI) at an optimum pH of 3 by the fungus *A. niger* varied from 82.1 to 92.5 %, *A. flavus* varied from 80.6 to 89.8 %, *A. fumigatus* varied from 79.8 to 85.4 %, *A. nidulans* varied from 77.8 to 81.6 %, *A. heteromorphus* varied from 72.4 to 76.3 %, *A. foetidus* varied from 68.8 to 72.6 %, *A. viridinutans* varied from 64.3 to 67.8 % (Table

4). The maximum removal percentage of Cr(VI) in a tannery industry wastewater by the isolated fungi species (in terms of F1-F7) at an optimum pH of 3, optimum contact time (incubation time) of 7 days with the biomass of 1.0 g of fungi species and an initial Cr(VI) concentration of 290 mg/L is in the order of *A. niger* [S1] > *A. flavus* [S4] > *A. fumigatus* [S8] > *A. nidulans* [S13] > *A. heteromorphus* [S17] > *A. foetidus* [S15] > *A. viridinutans* [S19] (Table 4).

#### Effect of fungus biomass

The Table 5 represents the removal percentage of Cr(VI) against each fungus biomass of 1, 2, 3, 4 and 5 g with an initial Cr(VI) concentration of 290 mg/L, the incubation period of 7 days and at an optimum pH of 3.0 (obtained from Table 4). The uptake of Cr(VI) in a tannery industry wastewater increases up-to the fungi biomass of 4 g and decreases with increase fungi biomass of 5 g (Table 5). It may be observed from Table 5 that less numbers of positive charge available by the fungi biomass, is leading to less Cr(VI) ion removal in a tannery industry waster by an isolated fungi biomass of 1, 2 and 3 g. Higher the removal against the 4 g of each biomass is due to availability of more quantity of enzyme secretion, and availability

Table 5: Effect of fungus biomass on removal percentage of Cr(VI) in a tannery industry wastewater by isolated fungi from tannery industry wastewater contaminated soil against an optimum pH of 3, optimum incubation time of 7 days, and an initial Cr(VI) concentration of 290 mg/L

Samples codes	Fungi species	Percentage removal of Cr(VI)				
		Fungi biomass, 1.0 g	Fungi biomass, 2.0 g	Fungi biomass, 3.0 g	Fungi biomass, 4.0 g	Fungi biomass, 5.0 g
S1	<i>A. niger</i>	80.3	84.2	88.6	94.5	77.5
S2	<i>A. niger</i>	78.5	82.3	86.7	92.4	75.8
S3	<i>A. niger</i>	75.8	79.6	83.7	89.3	73.2
S4	<i>A. flavus</i>	77.5	80.5	84.8	91.2	74.0
S5	<i>A. niger</i>	74.1	76.9	81.0	87.2	70.8
S6	<i>A. niger</i>	71.6	74.4	78.3	84.3	68.4
S7	<i>A. flavus</i>	74.8	77.7	81.9	88.1	71.5
S8	<i>A. fumigatus</i>	74.6	78.0	81.5	87.1	71.8
S9	<i>A. flavus</i>	73.1	75.7	79.2	85.4	69.7
S10	<i>A. flavus</i>	71.3	73.9	77.2	83.3	68.0
S11	<i>A. fumigatus</i>	72.6	75.2	78.6	84.8	69.2
S12	<i>A. fumigatus</i>	70.1	72.6	75.9	81.9	66.8
S13	<i>A. nidulans</i>	72.3	74.9	78.3	84.5	68.9
S14	<i>A. nidulans</i>	69.3	71.7	75.0	80.9	66.0
S15	<i>A. foetidus</i>	65.8	69.8	72.4	76.3	64.2
S16	<i>A. foetidus</i>	62.3	66.1	68.5	72.2	60.8
S17	<i>A. heteromorphus</i>	70.0	74.2	77.0	81.1	68.3
S18	<i>A. heteromorphus</i>	67.6	71.7	74.5	78.4	66.0
S19	<i>A. viridinutans</i>	61.2	65.0	67.4	70.9	59.8
S20	<i>A. viridinutans</i>	59.1	62.8	65.2	68.5	57.8



of more positive charge on fungi species, which could be sufficient to biosorb the Cr(VI) ion present in a tannery industry wastewater. The decrease in reduced capacity by an isolated fungi species biomass of 5 g is due to more biomass of fungi species having same positive charges and as a result, more repelling nature of the fungi biomass, which leads to reduction of Cr(VI) ion by the isolated fungi species.

The Cr(VI) removal in a tannery industry wastewater of an isolated fungi species has shown similar trends as similar to Cr(VI) removal against pH and an optimum fungus biomass of all isolated fungi species found in this study is 4 g (Table 5). The removal percentage of Cr(VI) at an optimum fungus biomass of 4 g by the fungus *A. niger* varied from 84.3 to 94.5 %, *A. flavus* varied from 83.2 to 91.2 %, *A. fumigatus* varied from 81.9 to 87.1 %, *A. nidulans* varied from 80.9 to 84.5 %, *A. heteromorphus* varied from 78.4 to 81.1 %, *A. foetidus* varied from 72.2 to 76.3 %, *A. viridinutans* varied from 68.5 to 70.9 % (Table 5). The maximum removal percentage of Cr(VI) in a tannery industry wastewater by an isolated fungi species (in terms of F1-F7) at a fungus biomass of 4 g, optimum pH of 3 (Table 4), optimum contact time (incubation time) of 7 days, and an initial Cr(VI) concentration of 290 mg/L is in the order of *A. niger* [S1] > *A. flavus* [S4] > *A. fumigatus*

[S8] > *A. nidulans* [S13] > *A. heteromorphus* [S17] > *A. foetidus* [S15] > *A. viridinutans* [S19] (Table 5). The results of effect of fungus biomass against the order of removal of Cr(VI) in a tannery industry wastewater is similar to that of the results of the effect of pH .

#### Effect of initial Cr(VI) concentration

Table 6 represents the removal percentage of Cr(VI) in a tannery industry wastewater at an optimum pH of 3 (Table 4), an optimum each fungi biomass of 4 g (Table 5), and an incubation time of 7 days against the dilution ratio of 0, 1, 2, 3 and 4 (1 part of tannery industry wastewater : 0, 1, 2, 3 and 4 parts of deionized water). It may be observed from Table 6 that the uptake of Cr(VI) increases upto the dilution of 4 (less concentration). For a lesser dilution ratio (high concentration) of 0, 1, 2 and 3, the tolerance of isolated fungi species in high Cr(VI) concentration is less and mobility of  $\text{CrO}_4^{-2}$  and  $\text{Cr}_2\text{O}_7^{-2}$  is also less, as a result, less removal percentage of Cr(VI) in a tannery industry wastewater by an isolated fungi species. Whereas, for a less concentration of Cr(VI) (more dilution ratio of 4), the isolated fungi species could be tolerated and able to biosorb the more quantity of Cr(VI) ion in a tannery industry wastewater because of presence of more mobile nature of  $\text{CrO}_4^{-2}$  and  $\text{Cr}_2\text{O}_7^{-2}$ , which leads to

Table 6: Effect of initial Cr(VI) concentration in removal percentage of Cr(VI) in tannery industry wastewater by isolated fungi from tannery industry wastewater contaminated soil against an optimum pH of 3, optimum fungus biomass of 4.0 g and incubation period of 7 days

Samples codes	Fungi species	Percentage removal of Cr(VI)				
		Concentration, 290 mg/L (dilution ratio, 0)	Concentration, 145 mg/L (dilution ratio, 1)	Concentration, 72.5 mg/L (dilution ratio, 2)	Concentration, 36.25 mg/L (dilution ratio, 3)	Concentration, 18.125 mg/L (dilution ratio, 4)
S1	<i>A. niger</i>	82.7	85.6	88.2	92.8	96.3
S2	<i>A. niger</i>	80.8	83.7	86.2	90.7	94.1
S3	<i>A. niger</i>	78.0	80.9	83.3	87.9	91.8
S4	<i>A. flavus</i>	79.1	82.4	83.3	88.5	92.8
S5	<i>A. niger</i>	75.4	78.7	79.7	84.8	88.7
S6	<i>A. niger</i>	72.8	76.1	77.0	81.8	85.2
S7	<i>A. flavus</i>	76.4	79.6	80.5	85.9	90.2
S8	<i>A. fumigatus</i>	76.6	79.5	80.3	85.8	90.1
S9	<i>A. flavus</i>	74.2	77.7	77.9	83.2	87.9
S10	<i>A. flavus</i>	72.0	75.7	76.0	80.7	85.7
S11	<i>A. fumigatus</i>	73.5	77.1	77.3	81.7	85.3
S12	<i>A. fumigatus</i>	71.0	74.5	76.2	79.5	83.1
S13	<i>A. nidulans</i>	73.0	76.9	78.6	82.2	86.7
S14	<i>A. nidulans</i>	69.7	73.5	75.2	78.7	82.4
S15	<i>A. foetidus</i>	67.7	70.4	73.0	76.0	78.6
S16	<i>A. foetidus</i>	64.1	66.7	69.1	72.1	74.3
S17	<i>A. heteromorphus</i>	71.6	74.9	77.6	81.0	83.7
S18	<i>A. heteromorphus</i>	68.6	72.4	73.7	77.5	80.1
S19	<i>A. viridinutans</i>	63.2	65.5	68.9	71.1	74.4
S20	<i>A. viridinutans</i>	60.5	63.3	65.6	68.4	71.6

higher removal percentage of Cr(VI) in a tannery industry wastewater by an isolated fungi species.

The Cr(VI) removal in a tannery industry wastewater of an each isolated fungi species of this study has shown similar trend and the optimum dilution ratio found from this study is 4 (Table 6). The removal percentage of Cr(VI) at an optimum dilution ratio of 4 by the fungus *A. niger* varied from 85.2 to 96.3 %, *A. flavus* varied from 85.7 to 92.8 %, *A. fumigatus* varied from 83.1 to 90.1 %, *A. nidulans* varied from 82.4 to

86.7 %, *A. heteromorphus* varied from 80.1 to 83.7 %, *A. foetidus* varied from 74.3 to 78.6 %, *A. viridinutans* varied from 71.6 to 74.4 % (Table 6). As similar to the effect of pH and fungus biomass, maximum removal of Cr(VI) in a tannery industry wastewater by an isolated fungi species (in terms of F1-F7) at an optimum pH of 3 (Table 4), an optimum fungus biomass of 4 g (Table 5), an optimum contact time (incubation time) of 7 days and an initial Cr(VI) concentration of 18.125 mg/L (dilution ratio of 4) is in the order of *A. niger* [S1] > *A. flavus* [S4] > *A. fumigatus* [S8] > *A. nidulans* [S13] > *A. heteromorphus* [S17] > *A. foetidus* [S15] > *A. viridinutans* [S19] (Table 6).

It may be noted from this study that the removal percentage of Cr(VI) in a tannery industry wastewater by an isolated fungi species was higher than the previous studies (Fukuda *et al.*, 2008a; Shugaba *et al.*, 2012). The variation between previous studies and the present study is due to the factors such as incubation time, initial concentration, type of isolated medium and type of source solution. In addition, the presence of one or more types of tolerance strategies or resistance mechanisms developed by the isolated fungi species (Shazia *et al.*, 2012). The percentage reduction of Cr(VI) observed by previous researchers using different fungi species is due to type of Cr(VI) solution (either aqueous or industrial wastewater) used for the study, the competitive ions like sulphates and chlorides in a tannery industry wastewater, the concentration of coexisting metal ions like Cu, Ni, and Cd (Lowe *et al.*, 2003; Zafar *et al.*, 2007), and metal binding process by the amino groups of major fungal wall constituents, chitin and chitosan (Velizar *et al.*, 2013).

*Aspergillus* sp. N2 decreased the Cr(VI) concentration to a virtually undetected level after 192 h growth in a medium containing 60 mg/L of Cr(VI) and at near neutral pH (Fukuda *et al.*, 2008b), but the *Aspergillus* sp. N2 able to reduce only 75 % of Cr(VI) concentration of 50 mg/L aqueous solutions at near

neutral pH (Acevedo-Aguilar *et al.*, 2008). The isolated fungi *Aspergillus* sp. N2, *Penicillium* sp. N3 from the chrome deposit was able to reduce the 70 % and 60 % respectively, with an initial concentration of 100 mg/L aqueous solution at the pH of 3.0 (Acevedo-Aguilar *et al.*, 2008). The mechanism of Cr(VI) reduction by *Aspergillus* sp. N2 is an enzymatic reduction (Fukuda *et al.*, 2008a) Furthermore, *A. niger* and *A. parasiticus* species were reduced the Cr(VI) concentration of 96.3 % and 91.6 % respectively from an initial concentration of 20 mg/L in 72 h (Shugaba *et al.*, 2012).

The fungi species such as *A. flavus* and *A. niger*, *Fusarium* sp. were more tolerant to chromium content (Shazia *et al.*, 2012). Similarly, the tolerance potential of *A. niger*, *A. flavus*, *A. fumigatus* were isolated from sewage contaminated agriculture soil of Kasur and industrial wastewater (Shazia *et al.*, 2013). The isolated fungi species were used to reduce chromium concentration in an aqueous solution and the degree of tolerance of fungi species was measured by minimum inhibitory concentration (Shazia *et al.*, 2013).

This study found that *A. niger* removed maximum Cr(VI) removal in a tannery industry wastewater than other fungi species. The results of this study are exhibiting the same as that of previous studies conducted on reduction of Cr(VI) in a tannery industry wastewater and an aqueous solution (Fukuda *et al.*, 2008a,b; Price *et al.*, 2001; Shazia *et al.*, 2009, 2012). Furthermore, *A. niger* has also shown a high level of tolerance to other tested toxic metals like Zn, Pb, Ni and Cd by *Fusarium* sp., and *Penicillium* sp. (Price *et al.*, 2001; Shazia *et al.*, 2009). The segregation of enzymes and development of molecular mechanisms is the main key properties of *Aspergillus* sp. to reduce the Cr(VI) ion in a tannery industry wastewater than other fungi species. The degradation potential or the metal binding capacity of *Aspergillus* sp. is fast during the fermentation process than other fungi species has contributed to the reduction of Cr(VI) ion in a tannery industry wastewater (Podgorskii *et al.*, 2004; Popa *et al.*, 2003; Xu *et al.*, 2012). Similarly, fungal wall constituents such as amino acids, chitin and chitosan (Velizar *et al.*, 2013) are also able to reduce Cr(VI) in a tannery industry wastewater.

#### Validation of experiment

Validation / Verification of experiments are required to verify the finding of observed results of the experimental investigation is stable and / or regular.

The most of the researchers have verified the experimental findings through replication (Radder 1992). This study used the baseline experiment (effect of pH, biomass and concentration) as replication (reputation of the same modality) and is used to check the degree of similarity between the experiments at optimum values of pH, biomass and concentration on removal of Cr(VI) in a tannery industry wastewater using different fungi species with the new experiment (separate experiment) conducted against the optimum values of pH, biomass and concentration on removal of Cr(VI) in an aqueous solutions.

In order to verify (simulate / repeat) the effect of pH, biomass and dilution ratio on reducing the Cr(VI) in a tannery industry wastewater by an isolated fungi species for any conditions, a separate experiment was conducted with an optimum pH of 3, optimum contact time (incubation time) of 7 days, optimum biomass (isolated fungi species) of 4.0 g of each fungi species and an aqueous Cr(VI) solution of concentration 18.125 mg/L. The concentration of 18.125 mg/L of Cr(VI) in an aqueous solution was prepared by dissolving known quantity of potassium dichromate in deionized water and kept in a volumetric flask of 500 mL. In order to develop color, added known quantity of 1, 5 Diphenyl carbazide to the aqueous solutions, mix well and allow the solutions to stand for 10 min. Then the absorbance measurements were performed at the wavelength of 542 nm using the Elico-UV spectrophotometer. The

maximum removal percentage of Cr(VI) in a tannery industry wastewater and an aqueous solution by an isolated fungi species at the optimum values of selected parameters is shown in Fig. 4.

The results (Fig. 4) showed that maximum removal percentage of Cr(VI) in an aqueous solution by an isolated fungi species at the optimum conditions (97.4 %) is greater than the results of Cr(VI) removal in a tannery industry wastewater (96.3 %) by an isolated fungi species. The order of maximum removal of Cr(VI) in an aqueous solution by an isolated fungi species at an optimum condition is *A. niger* > *A. flavus* > *A. fumigatus* > *A. nidulans* > *A. heteromorphus* > *A. foetidus* > *A. viridinutans*. It may be noted that the order of removal of Cr(VI) in an aqueous solution is similar to that of the order of removal of Cr(VI) in a tannery industry wastewater by an isolated fungi species. The high value obtained in an aqueous solution than in a tannery industry wastewater is due to there are no competitive ions present in aqueous solution than in a tannery industry wastewater, where competitive ions like chloride, sulphate (Lowe *et al.*, 2003; Zafar *et al.*, 2007; Sivakumar 2015), TDS, TS, BOD, COD are presented (Sivakumar, 2015).

Further, to check the degree of consistency on removal of Cr(VI) using *A. niger* (S1), the obtained maximum removal percentage of Cr(VI) using isolated *A. niger* (S1) with an optimum pH of 3, optimum contact time (incubation time) of 7 days, optimum biomass

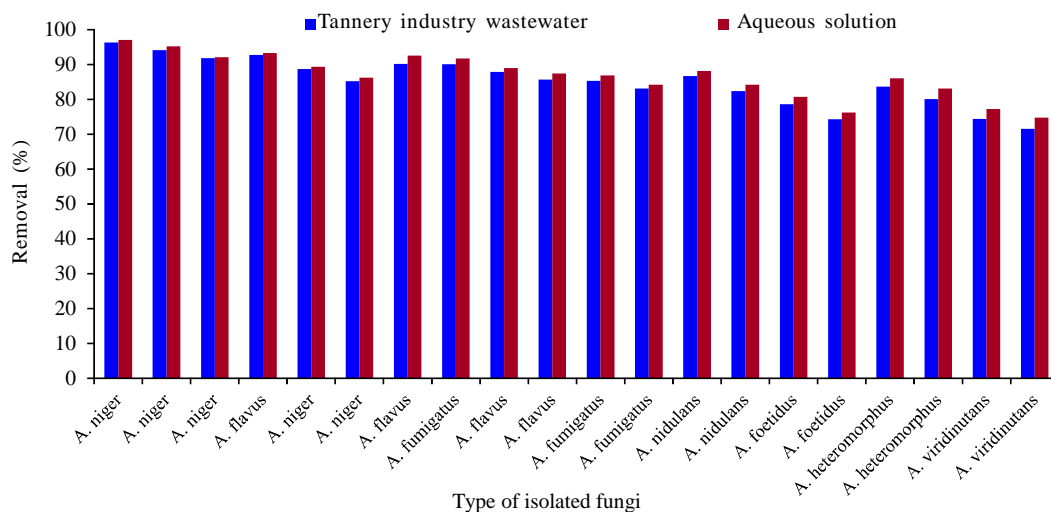


Fig. 4: The maximum removal percentage Cr(VI) in a tannery industry wastewater and in an aqueous solution by an isolated fungi with an optimum pH of 3, optimum incubation time of 7 days, optimum fungus biomass of 4.0 g and an initial Cr(VI) concentration of 18.125 mg/L

(isolated fungi species) of 4.0 g of each fungi species and an aqueous Cr(VI) solution of concentration 18.125 mg/L was verified with the other physico-chemical parameters viz., TDS, TS, COD, BOD, and  $\text{SO}_4^{2-}$  in a tannery industry wastewater using the same isolated *A. niger* (S1). The results of maximum reduction of various parameters TDS, TS, COD, BOD, and  $\text{SO}_4^{2-}$  along with reduction of Cr(VI) in a tannery industry wastewater using isolated *A. niger* (S1) at optimum values of pH, contact time, biomass and concentration are presented in Table 7. The other isolated fungi species results were not presented in this study, because *A. niger* (S1) produced maximum reduction of Cr(VI) in a tannery industry wastewater than other fungi species. From the validation experiments of other parameters in a tannery industry wastewater, it was

Table 7: The maximum reduction of Cr(VI) and other physico-chemical parameters in a tannery industry wastewater by isolated *A. niger* (S1) with an optimum pH of 3, optimum contact time (incubation time) of 7 days, optimum biomass (isolated fungi species) of 4.0 g of each fungi species and an aqueous Cr(VI) solution of concentration 18.125 mg/L

Parameters	Initial	Final	Removal (%)
Cr(VI)	290 mg/L	10.7 mg/L	96.31
TDS	11890 mg/L	915 mg/L	92.30
TS	15650 mg/L	344 mg/L	97.80
COD	7865 mg/L	1211 mg/L	84.60
BOD	5325 mg/L	553 mg/L	89.62
$\text{SO}_4^{2-}$	792 mg/L	45 mg/L	94.32

Table 8: Minimum incubatory concentration (MIC) and growth rate of isolated fungi species

Samples codes	Fungi species	MIC (mg/L)	Growth rate (cm)
S1	<i>A. niger</i>	590	0.040
S2	<i>A. niger</i>	610	0.047
S3	<i>A. niger</i>	640	0.053
S4	<i>A. flavus</i>	530	0.035
S5	<i>A. niger</i>	660	0.058
S6	<i>A. niger</i>	680	0.061
S7	<i>A. flavus</i>	560	0.043
S8	<i>A. fumigatus</i>	480	0.033
S9	<i>A. flavus</i>	600	0.051
S10	<i>A. flavus</i>	640	0.058
S11	<i>A. fumigatus</i>	560	0.046
S12	<i>A. fumigatus</i>	620	0.054
S13	<i>A. nidulans</i>	420	0.038
S14	<i>A. nidulans</i>	580	0.048
S15	<i>A. foetidus</i>	340	0.036
S16	<i>A. foetidus</i>	480	0.039
S17	<i>A. heteromorphus</i>	370	0.037
S18	<i>A. heteromorphus</i>	560	0.043
S19	<i>A. viridinutans</i>	310	0.031
S20	<i>A. viridinutans</i>	360	0.034

found that the order of maximum removal of other parameters in a tannery industry wastewater by an isolated fungi species is in the order of *A. niger* > *A. flavus* > *A. fumigatus* > *A. nidulans* > *A. heteromorphus* > *A. foetidus* > *A. viridinutans* (data not shown).

The validation results (Fig. 4 and Table 7) indicated that the optimum values found from this study were reproducing capability for analyzing aqueous solution and in any type of industrial wastewaters.

#### Minimum incubatory concentration and tolerance index

In order to evaluate the tolerance potential among isolated fungi species, the prepared Potato Dextrose Agar (PDA) medium was amended with various concentrations of chromium solution ( $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ). The PDA plates of each sample (from S1 to S20) were supplemented 100 to 1000 mg/L with an increment of 100 mg/L of chromium concentration solution at an optimum pH of 3.0. Further, to know the exact tolerance potential of the isolated fungi species, the PDA plates of each sample (from S1 to S20) were supplemented with a narrow concentration of increment of 10 mg/L of Cr(VI) solution.

The different concentration of Cr(VI) amended PDA mediums were inoculated with a drop of the isolated fungi spore suspension. The plates were incubated at 28 °C for an optimum contact time of 7 days. At the end of 7 days contact time, the growth of fungi in terms of radial extends in a Cr(VI) amended PDA mediums from the point of inoculation was monitored. The Cr(VI) metal tolerance against the all isolated fungi species was measured by observing minimum inhibitory concentration (MIC) and tolerance index (TI) (Shazia *et al.*, 2009, 2012; Zafar *et al.*, 2007). The exact MIC for all isolated species was identified by conducting a separate experiment with a narrow concentration of increment of 10 mg/L of Cr(VI) solution. The results of the MIC for each fungi species are presented in Table 8.

From Table 8, it may be observed that the maximum growth diameter for the *A. niger* was found to vary from 0.040 to 0.061 m with the MIC range from 590 to 680 mg/L. The maximum growth diameter was found to vary from 0.035 to 0.058 m, 0.033 to 0.054 m, 0.038 to 0.048 m, 0.037 to 0.043 m, 0.036 to 0.039 m and 0.031 to 0.034 m respectively for the fungi species such as *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans*, which was observed

against the MIC range from 530 to 640 mg/L, 480 to 620 mg/L, 420 to 580 mg/L, 370 to 560 mg/L, 310 to 480 mg/L and 140 to 360 mg/L respectively. There is no much growth diameter range difference observed below the Cr(VI) concentration of 590, 530, 480, 420, 370, 340 and 310 mg/L for the fungi species *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. Viridinutans* respectively. Further, beyond the Cr(VI) concentration of 680, 640, 620, 580, 560, 480 and 360 mg/L for the fungi species *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans* respectively, the growth of each isolated fungus species in a different concentration of Cr(VI) amended PDA mediums was started reducing (Table 8).

The MIC was observed to be 300 and 600 mg/L for the removal of chromium using *Aspergillus* and *Penicillium* fungi species respectively (Iqbal *et al.*, 2006). The MIC was found to be 1000, 800 and 1000 mg/L for the *A. niger*, *A. flavus* and *A. Versicolor* fungi species respectively against chromium reduction in an aqueous solution (Shazia *et al.*, 2012). Similarly, the MIC was found to be 381, 900 and 293 mg/L respectively for the reduction of Cu, Cd and Ni metal ions in an aqueous solution using *Aspergillus sp.* (Shazia *et al.*, 2009). *Aspergillus sp.* was more tolerant to Cu, Cd and Ni metal ions than other fungi species such as *Curvularia*, *Acrimonium* and *Pithyum* (Akhtar *et al.*, 2013). The variation of MIC between the isolated fungi species was due to the fast growing nature of

species and metal dependent (Iqbal *et al.*, 2006) and the medium (contaminated water, wastewater and soil environment) from which fungi species were isolated (Akhtar *et al.*, 2013).

The MICs of 590 and 530 mg/L was observed from this study for the fungi species *A. niger* and *A. flavus* respectively, which are quite opposite to that of the results observed in the previous study (Shazia *et al.*, 2013), where *A. flavus* and *A. niger* were tolerated with the MIC of 400 and 200 mg/L respectively. The variation between the present study and previous study (Shazia *et al.*, 2013) is due to *A. flavus* was more tolerated in the sewage industrial wastewater contaminated soil (Shazia *et al.*, 2013) and *A. niger* is more tolerated in tannery industry wastewater contaminated soil (this study). The results of the present study are closed to the results of the other study (Zafar *et al.*, 2007).

The tolerance potential of various fungi species like *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans* against the reduction of Cr(VI) in a tannery industry wastewater is expressed by tolerance index (TI). The metal TI is given in Eq. 2.

$$TI = \frac{R_t}{R_{ut}} \quad (2)$$

where,  $R_t$  is the radial growth (m) of isolated fungi species of the treated colony with Cr(VI) and  $R_{ut}$  is the

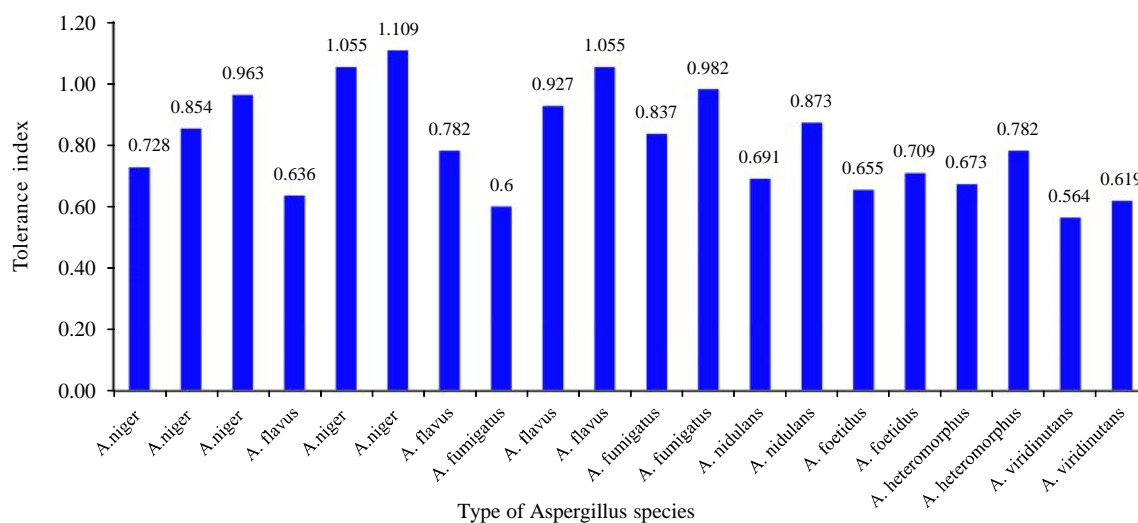


Fig. 5: Tolerance index (TI) of Aspergillus species

radial growth (m) of isolated fungi species of the untreated colony (control sample).

The TI calculated using the Eq. (2) for the isolated fungi species like *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans* is shown in Fig. 5. From Fig. 5, it may be observed that the TI was varied from 0.728 to 1.109, 0.636 to 1.055, 0.600 to 0.982, 0.691 to 0.873, 0.655 to 0.709, 0.673 to 0.782 and 0.564 to 0.619 for the fungi species such as *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans* respectively. It is also observed from Fig. 5 that *A. niger* and *A. flavus* were high tolerance to high Cr(VI) concentration, *A. fumigatus*, *A. nidulans* and *A. foetidus* were moderate tolerance to high Cr(VI) concentration, and *A. heteromorphus* and *A. viridinutans* were more sensitive to high Cr(VI) concentration.

The TI value of the isolated fungi species *A. niger* and *A. flavus* is greater than 1 indicated that the Cr(VI) may be induced the enzymatic processes of the isolated fungi species *A. niger* and *A. flavus*, which in turn reflected in high growth rate in PDA plates supplemented with Cr(VI) solution of different concentration than in a control PDA plate. From Fig. 5, it may be observed that *A. niger* showed the highest TI. The highest TI by *A. niger* is due to the presence of peroxidase enzyme that act as a catalyze, reacts quickly between the selected isolated species and Cr(VI) ion, which in turn used to reduce the Cr(VI) concentration in a tannery industry wastewater than other fungi species.

The previous studies indicated that the TI of Cr(VI) varied from 0.60 to 1.30 for *A. flavus*, 0.68 to 1.50 for *A. niger*, and 0.16 to 1.37 for *A. fumigatus* and the TI of Pb(II) metal ion varied from 0.16 to 0.35 for *A. flavus*, 0.25 to 0.82 for *A. niger*, and 0.17 to 0.30 for *A. fumigatus* (Shazia *et al.*, 2013). Among the isolated *Aspergillus* species, *A. flavus* and *A. niger* were found to be more tolerant against Pb(II) than *A. fumigatus*. On the other hand, *A. fumigatus* showed high tolerance against Cr(VI) than *A. flavus* and *A. niger*. Similarly, TI varied from 0.60 to 2.00 for copper, 0.40 to 1.40 for nickel and 0.3 to 0.6 for cadmium ions by *A. flavus* and TI varied from 0.80 to 1.30 for copper, 0.30 to 0.80 for Ni and 0.4 to 0.6 for Cd ions by *A. niger* (Akhtar *et al.*, 2013). The TI results of this study are also very close to the results of those reported by previous studies (Akhtar *et al.*, 2013; Shazia *et al.*, 2012, 2013; Zafar *et al.*, 2007).

It was found from the study that growth rate and TI of all isolated fungi species was high at lower concentrations and low at higher concentrations (no growth stage). The tolerance between isolated fungi species of tannery industry wastewater contaminated soil against the Cr(VI) ion in a tannery industry wastewater is in the order of *A. niger* > *A. flavus* > *A. fumigatus* > *A. nidulans* > *A. heteromorphus* > *A. foetidus* > *A. viridinutans* for both MIC and TI values. Furthermore, it may be found that *A. niger* is better to more tolerate in higher Cr(VI) concentration than other fungi species. The better tolerance is due to the development of the more physiological adaptation mechanism by *A. niger* in an elevated Cr(VI) concentrations than other fungi species. The tolerance resistance differences between the isolated fungi species are due to the mechanism of resistance of each fungus species (Ezzouhri *et al.*, 2009; Shazia *et al.*, 2013). Further, variations between the different fungi species depend on the isolate than the site of its isolation (Price *et al.*, 2001; Shazia *et al.*, 2009; Zafar *et al.*, 2007).

This study identified that the isolated fungi species such as *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans* of this study are very viable and useful for bioremediation of chromium contaminated water, wastewater and soil. Generally, fungi species are used to remove the hydrocarbon in the industrial pollution water and soil (Potin *et al.*, 2004; Shazia *et al.*, 2013). But this study suggests that the isolated fungi species of this study exhibits considerable tolerance towards Cr(VI) and can become dominant microorganisms, which can be used for removing other heavy metal ions in the some other contaminate water, wastewater and soil environment.

#### Biosorption model

The assumptions made for developing this biosorption model are (i) the temperature and pressure of the experimental solution are equal to atmospheric temperature and pressure, (ii) the rate of removal of ion in the experimental solution depends on the type of fungi isolated from type of sources, (iii) after attainment of equilibrium, the fungi species seize not too active in pollution control and (iv) the operation time (degradation time) is finite limit, within this finite limit, maximum removal of ion in the experimental solution could be achieved.

Let 'C' is the Cr(VI) concentration (mg/L) in a tannery industry wastewater by an isolated fungi species at time 't' (days). The degradation rate of Cr(VI) in a tannery industry wastewater by an isolated fungi species upto equilibrium time ( $t_{max}$ ) is directly proposed to the biosorption potential of isolated fungi species, to reduce between initial concentration and equilibrium concentration of Cr(VI) in a tannery industry wastewater for the time 't'. Mathematically, the biosorption model may be written as Eqs. 3 and 4:

$$\frac{dC}{dt} \propto -C \tag{3}$$

$$\frac{dC}{dt} = -K_c C \tag{4}$$

where  $K_c$  is the biosorption rate constant and negative sign (−) indicates, reduction of Cr(VI) is degrading by fungi species. After integrating the Eq. 4, becomes

$$C = C_i \exp(K_c t) \tag{5}$$

where  $C_i$  is the initial concentration of Cr(VI) in a tannery industry wastewater and the value of  $K_c$  is positive if curve increases and negative if curve decreases. From Eq. 5,  $K_c$  is calculated by

$$K_c = -\frac{\left(\ln\left(\frac{C}{C_i}\right)\right)}{t} \tag{6}$$

For the time interval  $t_1, t_2, t_3, \dots, t_N$ , the Eq. 6 becomes

$$K_{ci} = -\frac{\left(\ln\left(\frac{C_i}{C_i}\right)\right)}{t} \tag{7}$$

where 'i' = 1, 2, 3, .....N days

The mean  $K_c$  value may be calculated by

$$K_c = -\frac{\sum_1^N K_{ci}}{N} \tag{8}$$

The biosorption rate constant ( $K_c$ ) value is used to predict the biosorption model equation for all isolated fungi species of this study, which in turn used to find the Cr(VI) reduction or degradation in a tannery industry wastewater at any time 't' and upto the equilibrium time ' $t_{max}$ '. The biosorption rate constant ( $K_c$ ) of each isolated fungus is presented in Table 9.

*Validation of biosorption model*

To validate and to know the best fit of the proposed biosorption model of this study to the experimental data on removal of Cr(VI) in a tannery industry wastewater by all isolated fungi species, the proposed model is assessed by the coefficient of determination ( $R^2$ ) and Chi-square error function ( $X^2$ ). The  $R^2$  obtained

Table 9: The biosorption rate constant, coefficient of determination and Chi-square values for the degradation of Cr(VI) in a tannery industry wastewater by an isolated fungi species

Samples codes	Fungi species	The mean $K_c$ value	$\chi^2$ value	$R^2$ value
S1	<i>A. niger</i>	0.4593	0.0125	0.9938
S2	<i>A. niger</i>	0.4040	0.0165	0.9930
S3	<i>A. niger</i>	0.3570	0.0178	0.9928
S4	<i>A. flavus</i>	0.3755	0.0219	0.9892
S5	<i>A. niger</i>	0.3112	0.0187	0.9922
S6	<i>A. niger</i>	0.2727	0.0136	0.9938
S7	<i>A. flavus</i>	0.3314	0.0148	0.9908
S8	<i>A. fumigatus</i>	0.3303	0.0182	0.9892
S9	<i>A. flavus</i>	0.3016	0.0119	0.9924
S10	<i>A. flavus</i>	0.2774	0.0105	0.9933
S11	<i>A. fumigatus</i>	0.2738	0.0206	0.9884
S12	<i>A. fumigatus</i>	0.2538	0.0214	0.9841
S13	<i>A. nidulans</i>	0.2840	0.0445	0.9701
S14	<i>A. nidulans</i>	0.2450	0.0545	0.9674
S15	<i>A. foetidus</i>	0.2182	0.0567	0.9697
S16	<i>A. foetidus</i>	0.1933	0.0357	0.9786
S17	<i>A. heteromorphus</i>	0.2549	0.0331	0.9785
S18	<i>A. heteromorphus</i>	0.2290	0.0246	0.9809
S19	<i>A. viridinutans</i>	0.1943	0.0109	0.9933
S20	<i>A. viridinutans</i>	0.1771	0.0062	0.9949

from the regression equation is the most common method used for assessing the association between two variables. The  $R^2$  obtained from the regression fit of biosorption model values and for removing Cr(VI) reduction or degradation in a tannery industry wastewater at any time 't' and upto the equilibrium time ' $t_{max}$ ' by each isolated fungus is given in Table 9.

From Table 9, it may be found that the  $R^2$  of the degradation model varied between 0.9674 and 0.9949 (> 95 % confidential interval) for Cr(VI) reduction or degradation in a tannery industry wastewater at any time 't' and upto equilibrium time ' $t_{max}$ ' by all isolated fungi species (sample code from S1 to S20). The use of  $R^2$  values is applicable only for the existing relationship between the selected depended variables. Though, the  $R^2$  values show greater than 95 % confidence intervals,

the  $R^2$  values is for the relationship between contact time (incubation time) and removal percentage of Cr(VI) in a tannery industry wastewater of the degradation model by an isolated fungi species *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans*.

Thus, the proposed biosorption model is also validated with Chi-square error function. The use of same abscissa and ordinate between the model and the experimental data fit is the advantage of using Chi-square error function. Further, Chi-square error function is used both model and experimental data for the percentage error calculation. If the model result is very close to the experimental result, the Chi-square value would be small, otherwise, it would be large. The Chi-square error function equation is given in Eq. 9.

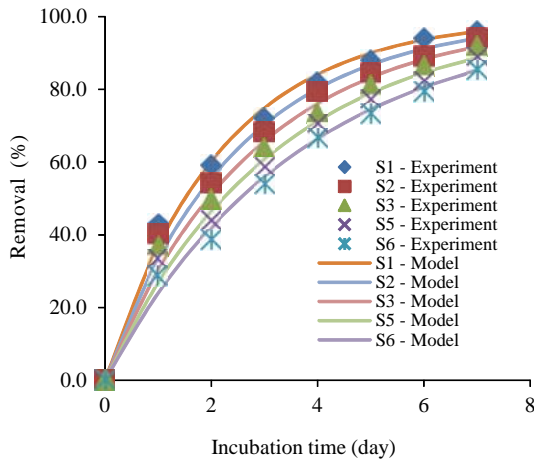


Fig. 6(a): Comparison of experimental and model values by *A. niger*

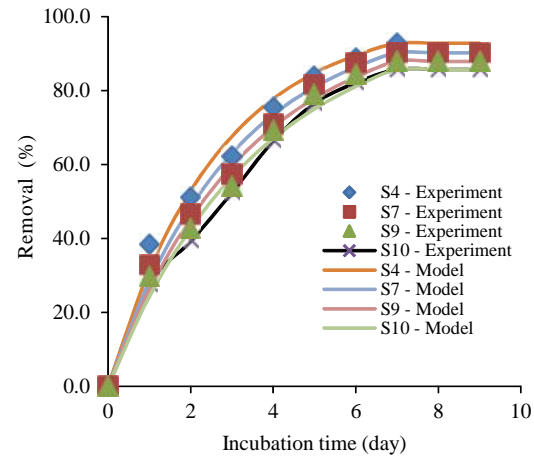


Fig. 6(b): Comparison of experimental and model values by *A. flavus*

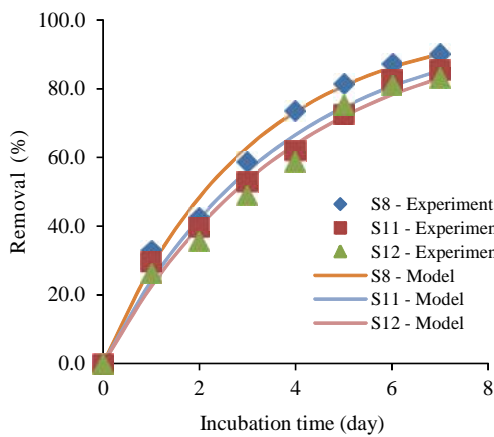


Fig. 6(c): Comparison of experimental and model values by *A. fumigatus*

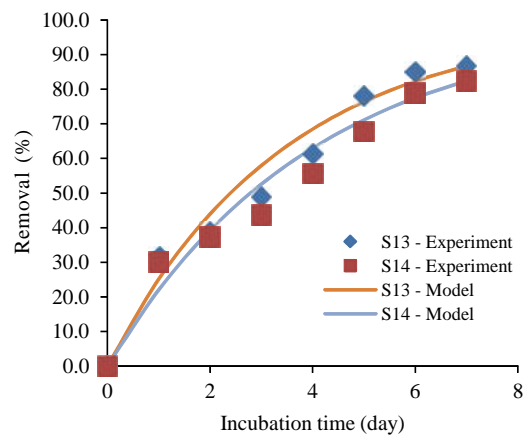


Fig. 6(d): Comparison of experimental and model values by *A. nidulans*



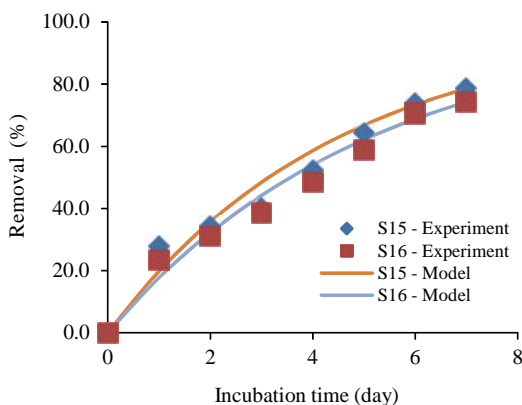


Fig. 6(e): Comparison of experimental and model values by *A. foetidus*

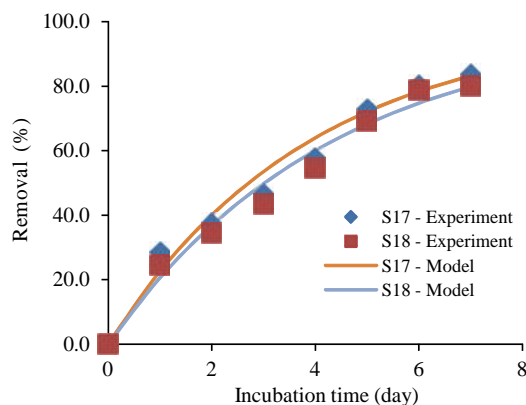


Fig. 6(f): Comparison of experimental and model values by *A. heteromorphus*

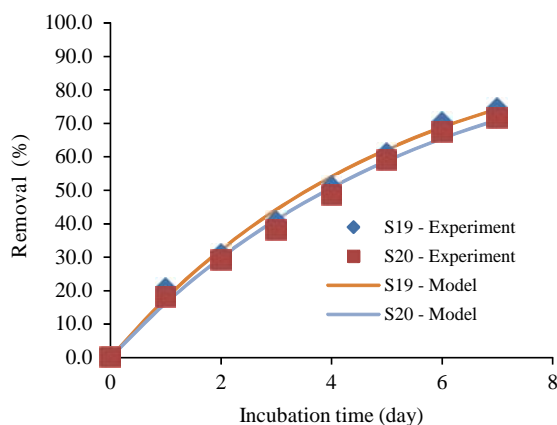


Fig. 6(g): Comparison of experimental and model values by *A. viridinutans*

Fig. 6 (a to g): The comparison of experimental observed values with biosorption model calculated values for different incubation time with an optimum pH of 3, optimum biomass (isolated fungi species) of 4.0 g of each fungus and optimum Cr(VI) concentration in a tannery industry wastewater of 18.125 mg/L

$$\chi^2 = \sum \frac{(C_E - C_M)^2}{C_M} \quad (9)$$

where  $C_E$  and  $C_M$  are observed Cr(VI) concentration (mg/L) in a tannery industry wastewater treated with isolated fungi species and calculated Cr(VI) concentration (mg/L) using the degradation model respectively at any time 't' (days).

The comparison of experimentally observed values and the biosorption model calculated values for different incubation time with an optimum pH of 3, optimum biomass (isolated fungi species) of 4.0 g of each fungus and optimum Cr(VI) concentration in a tannery industry wastewater of 18.125 mg/L is shown in Fig. 6 (Figs. 6a-6g). From Fig. 6 (Figs. 6a-6g), it may be observed that the percentage removal of Cr(VI) in a

tannery industry wastewater by an isolated fungi species *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. heteromorphus*, *A. foetidus*, and *A. viridinutans* for the biosorption model values are very close to the percentage removal observed from the experimental investigations. Furthermore, the comparison of biosorption model values with the experimental data obtained at an optimum pH, contact time (incubation time), biomass and optimum Cr(VI) concentration in a tannery industry wastewater of 18.125 mg/L using Chi-square error function is presented in Table 9. From Table 9, it may be observed that the Chi-square error function values varied from 0.0062 % to 0.0567 % for Cr(VI) reduction or degradation rate in a tannery industry wastewater at any time 't' and upto equilibrium

time ' $t_{max}$ ' by all isolated fungi species (sample code from S1 to S20). Based on  $R^2$  value of the biosorption model and the minimum error percentage of  $x^2$  value between the model calculations and experimental observations, the proposed biosorption model is very much useful for predicting the trend of biosorption potential of an isolated fungi species or removal of Cr(VI) in a tannery industry wastewater by all isolated fungi species. This biosorption model (Eq. 5) is also useful for identifying the removal potential of any organisms used for treatment of any contaminated water and soil at any time interval or reduction of any ion present in the any experimental solution at any time interval.

## CONCLUSION

The present study focused to isolate the different fungi species in a tannery industry wastewater contaminated soil, which in turn used to reduce the Cr(VI) concentration in a tannery industry wastewater. The ability of isolated fungi species *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. foetidus*, *A. heteromorphus* and *A. viridinutans* from a tannery industry wastewater contaminated site of Nagalkeni for reducing Cr(VI) concentration in a tannery industry wastewater were monitored against the various pH, fungi biomass and dilution ratio. The maximum percentage reduction of Cr(VI) in a tannery industry wastewater by the fungus *A. niger* at an optimum pH of 3, optimum contact time (incubation time) of 7 days, optimum fungi species biomass of 4.0 g and with an initial Cr(VI) concentration of 18.125 mg/L (dilution ratio 4). The metal tolerance test confirmed that each isolated fungus species such as *A. niger*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. foetidus*, *A. heteromorphus* and *A. viridinutans* are able to remove the Cr(VI) concentration range between 310 and 680 mg/L. The order of maximum removal of Cr(VI) and other parameters in a tannery industry wastewater and an aqueous solution by an isolated fungi species is in the order of *A. niger* > *A. flavus* > *A. fumigatus* > *A. nidulans* > *A. heteromorphus* > *A. foetidus* > *A. viridinutans*. The proposed biosorption model in this study is used to simulate the experimental data on removal of Cr(VI) in a tannery industry wastewater by all isolated fungi species and the proposed model was assessed by the coefficient of determination ( $R^2$ ) and Chi-square error function ( $X^2$ ). The  $R^2$  and values suggested that the proposed biosorption model is very

much useful for predicting the trend of degradation potential of an isolated fungi species. Long time exposure of soil fungi to Cr(VI) can lead to physiological adaptation or considerable modification of their microbial populations, increasing their activity and their number, and such changes may be associated with increased metal reduction capacity by all isolated fungi species of this study. This study suggested that one could select the type of fungi species, metal concentration level, selection of treatment period, quantity of biomass to be used, and pH level of the medium, to achieve the highest reduction of any ions from the contaminated water, wastewater and soil environment. In addition, further investigations are to be needed to identify the toxic metal tolerant fungi species in multi-metal water, wastewater and soil environment.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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