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Removal of Cr (III) from model solutions and a real effluent by *Phanerochaete chrysosporium* isolated living microorganism: equilibrium and kinetics

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ABSTRACT

Removal of Cr (III) was investigated using *Phanerochaete chrysosporium*-isolated living microorganism; pH, contact time, temperature and nutrients addition were examined. It was found that *P. chrysosporium* can tolerate up to 600 mg/L chromium solution. The optimal growth conditions of the biosorbent were found to be 35°C, 26 h contact time and pH = 5. In addition, a complex nitrogen substrate, yeast powder, was shown to be most efficient than a synthetic one, like di-hydrogen ammonium phosphate. High chromium removal (98%) was observed in these optimal growth conditions. Experimental data were found to follow a Langmuir isotherm model ($r^2 > 0.99$). Maximum sorption capacity for the present biosorbent was 213 mg/g according to the Langmuir isotherm model, namely significantly higher than the values reported in the literature, even for activated carbon. The fitting of experimental data onto kinetic models showed the relevance of the pseudo-second-order model ($r^2 > 0.99$) for Cr (III) sorption by *P. chrysosporium*. In addition, a real effluent was obtained from tanning factory and was treated to examine process feasibility on real effluents.

Keywords: Batch study; Cr (III); Biosorption; Growth rate; Equilibrium study

1. Introduction

Heavy metals are one of the main environmental pollutants and the main source of this pollution is industrial activities. Among the common heavy metals involved in environmental pollution, lead, cadmium, mercury, nickel, and chromium are reported [1,2]. Heavy metals are toxic and can accumulate in living organism tissues. Among them, chromium is a toxic metal, which is used in many industries such as textile dyeing, leather tanning, electroplating, metal finishing, and other activities [3–5]. Chromium exists in several oxidation states in the environment

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and industrial effluents. The most stable and common forms are the trivalent Cr (III) and the hexavalent Cr (VI) species [6,7]. Hexavalent chromium is known as 500–1,000 times more toxic than trivalent chromium due to its high solubility and significant mobility in the environment [3,8]. Several diseases such as epigastria, nausea, vomiting, and dermatitis are considered as chromium effect on human being [9]. Due to high toxicity of hexavalent chromium, its maximum level in aqueous environment is considered to be below 0.1 mg/L [10]. Tanning factory effluents contain high Cr (III) levels. Even if this type of chromium is less toxic and carcinogenic than the former, with time elapse it can be oxidized to Cr (VI) [4,11]. Its oxidation to the more carcinogenic and mutagenic Cr (VI) by MnO_2 in the environment or by some bacteria has been also reported [12,13]. In addition, it is toxic to fish for concentrations in water exceeding 5 mg/L [13,14]. Therefore, the removal of Cr (III) from effluents before their releasing is also an important environmental issue. For this purpose, in recent years researchers tested several methods such as chemical precipitation, ion exchange, biological process and others for the removal of Cr (III) and (VI) [15–17]. Among them, adsorption has received much attention to remove chromium ions, especially using activated carbon as an adsorbent. The economic issue and the need for regeneration of the used adsorbents led researchers to investigate other inexpensive and economic adsorbents for the removal of chromium [18,19]. Among the various adsorbents, an emerging and promising method appears to be the use of biomaterials (refer to biosorption). In recent years, this method has attracted an increasing attention for the removal of environmental pollutants; its main characteristic is the use of living or dead microorganisms [20]. Biomass from wetland or any other marsh area can be used for biosorption [21]. Hence, several researchers have used biosorption for the removal of heavy metals [22]. Based on chromium effect on environment and the numerous advantages of biosorption, the aim of this work was the seeking and the isolation of fungal microorganisms from tanning factory environment for the removal of trivalent chromium. Usually, dead cells are used for this purpose, while viable fungi are rarely used. Indeed, working with viable cells is more complex than the use of dead cells. Therefore, living isolated microorganisms have been tested in this work and growth conditions were optimized for the removal of chromium. In addition, attempts have been made to simulate the behavior occurring in real tanning wastewater.

2. Materials and methods

2.1. Sampling and isolation of relevant microorganisms

To seek and isolate sustainable microorganisms, samples from rawhide, tanning basin, and effluents discharged environment were taken. Soil samples were then incubated to seek for relevant microorganisms. Molasses (20 g molasses was mixed with 100 g soil sample) were used as culture medium and an antibiotic (chloramphenicol, 5 mg/L) was added to inhibit bacterial growth. Since the aim of work was the identification and the isolation of fungi, medium pH was adjusted to 5.5 by 1 N NaOH or H_2SO_4. Plates (containing molasses and soil sample) were incubated at 30˚C upon six days. Cultures revealed that various fungi can be found in the environment of tanning industry. All organisms that were found after plate cultures were compared with standard species obtained from the Iranian Center for Scientific and Industrial Research, and among them, a *P. chrysosporium* strain was selected for this work. The isolated fungal strain was separated and kept in stock culture at 4˚C for subsequent experiments.

2.2. Reproduction and preparation of biosorbent

After isolation of a resistant microorganism, the Potato Dextrose Agar (PDA) containing 300 g potato, 20 g glucose, 15 g agar, and 1 l distilled water was used as fungal reproduction culture. Fungal reactivation was done by adding some drop samples from the previous step (from molasses culture) to the appropriate culture medium (PDA culture). The culture was incubated at 30˚C for 6–7 days and was then taken and used for experiments. To determine fungal biomass, a given amount of culture was filtered, dried at 105˚C for 24 h, and then, the filter was weighed before and after drying.

2.3. Chemicals and instruments

All chemicals used in this work were of GR grade and obtained from Merck. 1 N NaOH or H_2SO_4 were used to adjust pH (model Sartorius Professional Meter PP-50). Since the main chromium ion in tanning industry is Cr (III), the chromium stock solution was prepared by adding an appropriate amount of Cr $(NO_3)_3$ onto de-ionized water. The chromium concentration in solution and also in biomass was determined by atomic absorption spectrometer (Model Alpha4-Chemtech, England) according to the Standard Methods for The Examination of Water and Wastewater section 3111B [23].

2.4. Batch experiments

Experiments were conducted in 250 ml beakers. Various parameters such as pH (3–8), contact time (0.5–38 h), nutrients addition (di-hydrogen ammonium phosphate and yeast powder), and temperature (10–45˚C) were investigated. To perform experiments, a specified amount of viable fungi was added to 100 ml chromium solution at various pH. The broth was set at 30˚C and stirred at 150 rpm for 38 h until equilibrium (stationary growth phase) was reached. It was then taken; filtered using 0.45 filters to remove biomass (Wathman) and final chromium concentration was measured using atomic absorption spectrometer. The removal efficiency was calculated using the following equation:

$$\mathrm{RE} = (C_0 - C_e) \times 100/C_0 \quad (1)$$

where RE(%) is the percentage of chromium removed at equilibrium time; C_o and C_e are the initial and equilibrium concentrations of chromium (mg/L), respectively.

The effect of nutrients addition was done by adding di-hydrogen ammonium phosphate (in the range 1–9 g/L) and yeast powder (in the range 0.5–2 g/L) onto 100 mL chromium solution. Then, 0.2 g of fungi was introduced into chromium solution containing appropriate nutrients and the solution pH was adjusted at 5. The mixture was then stirred at 150 rpm until stationary phase or maximum chromium removal efficiency was reached.

2.5. Kinetic modeling

Kinetic data were collected at various initial chromium concentrations and showed to be constant and equal to 26 (h) irrespective of the initial chromium concentration. 0.3 g/L of *P. chrysosporium* as dried mass was added onto 250 mL beaker and shaken at 150 rpm until reaching equilibrium. The solution pH and temperature were 5 and 35˚C, respectively. Samples were regularly taken, filtered and chromium concentration was determined as previously mentioned. Pseudo first-order-and pseudo-second-order kinetic models were used to investigate Cr (III) biosorption by the selected fungus.

The pseudo-first-order equation is generally expressed as follows:

$$\frac{\mathrm{d}q_t}{\mathrm{d}t} = k_1(q_e - q_t) \quad (2)$$

Integration of Eq. (2) at the boundary of $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, gives:

$$\mathrm{Log}\left(1 - \frac{q_t}{q_e}\right) = -\frac{k_1}{2.303}t \quad (3)$$

where q_e and q_t are the amounts (mg/g) of adsorbate at equilibrium and at time t (min) respectively; and k_1 is the rate constant (1/min). The linear plot of log $1 - q_t/q_e$ vs. t gives the k_1 from the slope of such plot. The general form of the pseudo-second-order kinetic is expressed by the following equation:

$$\frac{\mathrm{d}q_t}{\mathrm{d}t} = k_2(q_e - q_t)^2 \quad (4)$$

Integration of Eq. (4) at the boundary of $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$ and then rearrangement to a linear form gives:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \quad (5)$$

where k_2 is the rate constant (g/mg min). The value of k_2 and q_e can be determined from the intercept and the slope of the plot t/q_t vs. t, respectively [24,25].

2.6. Isotherm study

Adsorption is usually described through isotherms, namely and for a given temperature, the amount of adsorbate vs. the adsorbate concentration. Isotherm experiments were conducted in 250 mL beakers. About 0.5 g/L of adsorbent (this mass was considered after reaching stationary growth phase) was added onto chromium solution in the range of 120–1,080 mg/L. Samples were stirred at 150 rpm for three days at 35˚C. Experimental data obtained from equilibrium study were analyzed by means of Langmuir, Freundlich and Temkin isotherm models.

The non-linear form of the Langmuir isotherm for monolayer adsorption is:

$$q_e = q_m b C_e / 1 + bC_e \quad (6)$$

The linear form of the Langmuir isotherm is expressed as follows:

$$C_e l q_e = C_e/q_m + 1/q_m b \quad (7)$$

where q_e is the equilibrium amount of adsorbate (mg/g), C_e is the equilibrium concentration of adsorbate (mg/L), q_m is maximum adsorption capacity, and b is

the Langmuir constant. By plotting of C_e/q_e against C_e, one can calculate q_m and b from the slope and intercept, respectively. The important feature of the Langmuir model can be described based on the R_L parameter expressed as follows:

$$R_L = \frac{1}{(1 + bC_0)} \tag{8}$$

Adsorption is unfavorable for $R_L > 1$, linear for $R_L = 1$, favorable for $0 < R_L < 1$ and irreversible for $R_L = 0$.

The Freundlich equation was developed empirically, having no theoretical basis, and is useful for describing the sorption of ions by chemical adsorption and surface precipitation reactions. The non-linear form of the Freundlich equation is expressed as follows:

$$q_e = K_f C_e^{1/n} \tag{9}$$

where K_f and n are the Freundlich constants. A high value of K_f characterizes a high affinity of the adsorbate. For favourable adsorption, the value of the Freundlich constant (n) should be in the range of 1–10. The linear form of the Freundlich isotherm can be expressed as follows:

$$\log(q_e) = \log K_f + 1/n \log C_e \tag{10}$$

Linear plots of log q_e versus log C_e can confirm that sorption data follows a Freundlich model. The Freundlich constants (n and k_f) can be obtained from the slope and the intercept, respectively.

The Temkin isotherm is also available for heterogeneous adsorption of adsorbate on a surface. The non-linear form of the Temkin model is the following:

$$q_e = RT/b_1 \ln(k_t C_e) \tag{11}$$

By rearranging Eq. (11), one can present the linear form of the Temkin model as follows:

$$q_e = B_1 \ln(k_t) + B_1 \ln(C_e) \tag{12}$$

where $B_1 = RT/b_1$, b_1 is the adsorption heat (kJ/mol), k_t is the equilibrium binding constant (L/g) corresponding to maximum binding energy. A high value of b_1 shows a fast sorption of adsorbate at initial stage. Similarly, low value of k_t is related to weak bonding of adsorbate onto the medium. By plotting q_e vs. ln (C_e) one can deduce B_1 and k_t from the slope and the intercept of this curve, respectively.

2.7. Real sample tests

To investigate the applicability of the obtained data, the removal of trivalent chromium from a real solution was examined. The real sample was obtained from tanning industry located in the south region of Tehran (Tehran, Iran). The effluent characteristic is shown in Table 1. Real samples were taken every three days and the average values are collected in Table 1. Real sample was conducted without any treatment using the selected fungus in separate 250 ml beakers. About 0.8 g/L of *P. chrysosporium* was added onto the beakers containing real samples. Based on laboratory results, 7 and 1.5 g/L of yeast powder and di-hydrogen ammonium phosphate as nutrients were used, respectively. The samples were shaken at 150 rpm for 26 h and the amount of chromium removed was measured.

2.8. Desorption tests

Desorption tests were conducted using 1 N H_2SO_4, NaOH and HNO_3. A 0.3 g/L of viable fungus was added onto 250 mL chromium solution containing 240 mg/L chromium and shaken at 150 rpm until equilibrium was observed. Biomass was then extracted, introduced into 250 mL deionized water free from chromium and then shaken at 150 rpm until maximum chromium leakage was observed. The chromium concentration was then determined as previously mentioned. The desorbed chromium onto deionized solution was considered as extracellular adsorbed chromium. After desorption of chromium into deionized water, the biomass was extracted, dried at 105°C for 24 h, and the remained chromium in biomass was determined and considered as intracellular adsorbed chromium.

3. Results and discussion

3.1. Determination of fungal tolerance to chromium

The aim of this section was to investigate what concentration of chromium solution can prevent from fungal growth. In addition, it has been reported that two main routes are involved in the removal of heavy metals by viable microorganisms. Firstly, heavy metals can be removed by passive process or independent from cell activity (electrostatic bounding with cell surface). Secondly, heavy metals would be removed by bounding with exopolymeric substances which only occurs in viable body [26,27]. Therefore, maximum biomass concentration would occur at an optimal chromium level or maximum fungal tolerance to

Table 1
Composition of tanning house effluent

Parameters	Amount	Average	SD[a]
pH	3–3.5	3.26	0.5
Total organic carbon (mg/L)	27,000–41,000	34,400	5272.6
Total kjeldahl nitrogen (mg/L)	160–245	208	36.6
Phosphate (PO_4^{3-}), mg/L	65–118	87.8	20.6
Cr^{+3} (mg/L)	1,000–1,300	1,185	122.8

[a]Standard deviation.

chromium toxicity. Maximum growth corresponds to the maximal production of exopolymeric substances leading to optimum chromium removal, showing the advantage of viable cells over non-viable cells, which are therefore expected to be more efficient. For this purpose, various inoculation levels and various initial chromium concentrations were considered, without any added nitrogen source. From a stock solution containing 0.7 g/L based on dry cell weight, a range of volumes was considered to inoculate chromium solutions. The results are displayed in Fig. 1 and showed that irrespective of the inoculation level, maximum fungal tolerance was 600 mg/L of chromium. In the range 120–600 mg/L chromium had no reverse effect on fungal growth, while above 600 mg/L final biomass concentrations decreased drastically. In addition, maximum fungal growth was observed for 100 mL/L inoculum level (10%), which was therefore considered thereafter. Maximum tolerance to Cr (VI) by *Synechocystis* sp. BASO670 and *Synechocystis* sp. BASO672 were found to be 11.5 and 2.0 mg/L, respectively [28]. The lower tolerance of *Synechocystis* sp. BASO670 and *Synechocystis* sp. BASO672 may be attributed to the higher toxicity of Cr (VI) compared to Cr (III). Removal of Cr (III) by viable *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum has been studied, and maximum tolerance was found to be 100 mg/L [29]. *Bacillus subtilis* strains from Palar river basin have been used for the removal of Cr (III). In that work, five bacterial strains were used and showed that *B. subtilis* VITSCCr01 can tolerate Cr (III) concentration up to 1,500 mg/L [30]. In our previously published work [31], the removal of Cr (III) by *Aspergillus niger* and *Aspergillus oryzae* has been reported and maximum tolerance to chromium was observed to be 500 mg/L. This demonstrated that *P. chrysosporium* (the present work) has a higher tolerance to Cr (III) compared with *Synechocystis* sp. BASO670, *Synechocystis* sp. BASO672, *A. niger* and *A. oryzae* and a lower tolerance if compared with *B. subtilis* strains.

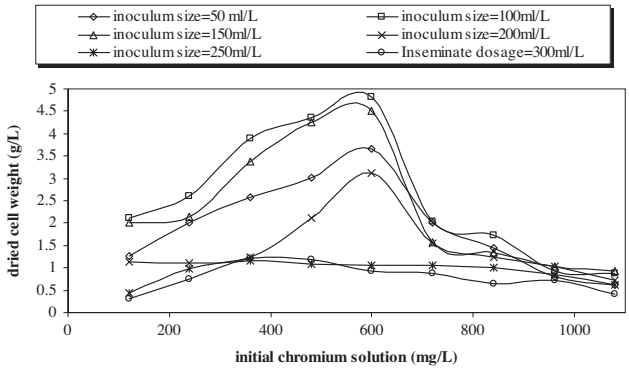

Fig. 1. Determination of fungal tolerance for chromium (T = 30 °C, contact time = 26 h, agitation speed = 150 rpm, pH=5 and inoculation levels in the range 50–300 mL/L).

3.2. Determination of the optimal pH

The pH has an important role regarding its effect on the one hand on microorganism growth, and on the other hand, on adsorption capacity, since the pH affects the surface charge of adsorbent and solute species [22,31]. Since in viable systems, the solute sorption rate is affected by the growth rate of microorganisms (leading to the production of exopolymeric substances) [27,32], determination of the optimal pH is needed and have been therefore investigated (Fig. 2). Final biomass concentration showed an optimal pH value of 5, namely at slightly acidic pH, as generally accepted for fungi. Adsorption was linked to final biomass amounts, since chromium removal increased for pH increasing from 3 to 5 and then decreased above pH 6; at pH 5, chromium removal was close to 85%. These results were statistically validated ($P_{value} < 0.05$). The low removal efficiency at lower pH (pH < 5) was due to the competition of hydrogen ion with chromium ion for sorption sites. As the pH increased from 3 to 5, the removal of trivalent chromium increased due to the strong linking between chromium uptake and the number of surface negative charges. At pH above 5, due to reduction in electrostatic attraction between the trivalent chromium ion and the adsorbent surface (reduction in positive sorption mechanism),

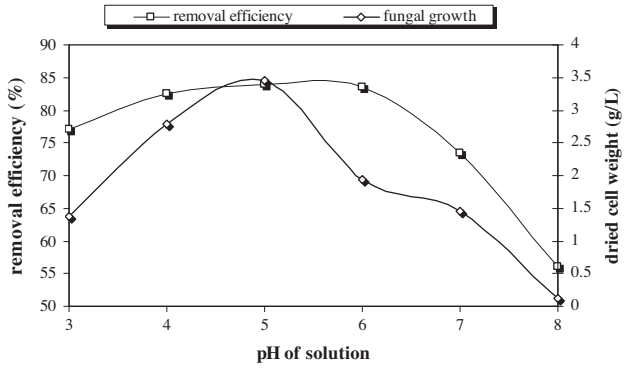

Fig. 2. Effect of pH on fungal growth and chromium removal (T = 30˚C, contact time = 26 h, agitation speed = 150 rpm, initial chromium solution = 240 mg/L).

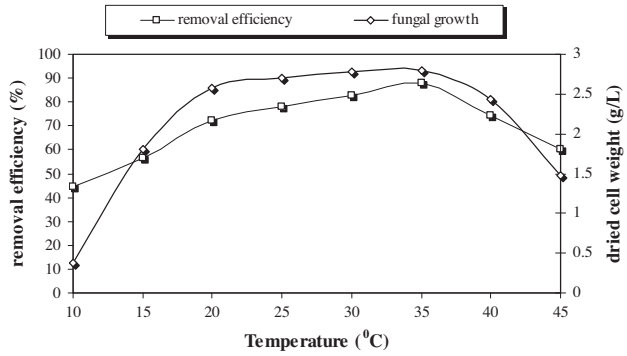

Fig. 3. Effect of the temperature on fungal growth and chromium removal (pH=5, contact time = 26 h, agitation speed = 150 rpm, initial chromium solution = 240 mg/L).

removal capacity decreased. In addition, in alkaline environment (pH=8), reduction in removal efficiency can be due to a reduction in metal solubility. On the other hand, maximum fungal mass was observed at the optimal pH value, which resulted in the maximal amount of exopolymeric substances, increasing removal efficiency [31,32]. Removal of Cr (VI) and Ni (II) by some viable *Aspergillus* species isolated from soil samples of an electroplating industry were investigated, showing an optimal pH of 5–5.2 for fungal growth and Cr (VI) and Ni (II) removal [9]. In the removal of Remazol Blue and Cu (II) by *Aspergillus versicolor* biomass, it has been reported that maximum removal percentage was observed to be at pH 5 [32]. In addition and in agreement with our results, maximum removal of copper (II) and cadmium (II) by free and immobilized biomass of *A. niger* was observed at 5.5 [33]; maximum removal of dyes from anaerobically digested spent wash by *A. niger* biomass, was observed at pH 5.5 [34]; and the removal of Pb (II) by *A. niger* and *A. versicolor* biomass was reported to be optimal at pH 5 [35]. In our previously published work, pH 5.3 was found to be the optimal value for the removal of Cr (III) from model solutions [31], showing that pH 5–5.5 was favorable to sorption of pollutants by fungi, especially heavy metals, in agreement with the present findings.

3.3. Determination of the optimal temperature

Temperature, another major parameter in adsorption, can affect the solute dissolution, as well as the reaction rate; it also affects microorganism growth. *P. chrysosporium* growth and chromium removal was investigated in a range of temperatures between 10 and 45˚C (Fig. 3). As shown in Fig. 3, final fungal biomass was optimal at 35˚C, even if in the range 20–40˚C temperature has only a rather low effect on growth. However, temperature showed a significant effect on chromium removal, which increased from 44 to 88% for temperatures increasing from 10 to 35˚C, with an optimal removal recorded as for growth at 35˚ C. From this, it can be concluded that the effect of temperature was preponderant over metal dissolution, showing that the latter was negligible in the range of temperatures considered. Many researchers used viable microorganisms for the removal of pollutants from wastewater and reported that temperatures in the range of 25–30˚C are suitable for growth of fungi. *A. niger* and *A. oryzae* growth and Cr (III) removal efficiency were shown to be optimal at 30˚C [31]. In addition, 30˚C has been reported to be the optimal temperature for the removal of reactive dyes and heavy metals by means of *A. versicolor* [32], as well as for the removal of Pb (II) by *A. niger* and *A. versicolor* [35].

3.4. Effect of the contact time

The effect of the contact time on fungal growth and removal efficiency was investigated (Fig. 4). As

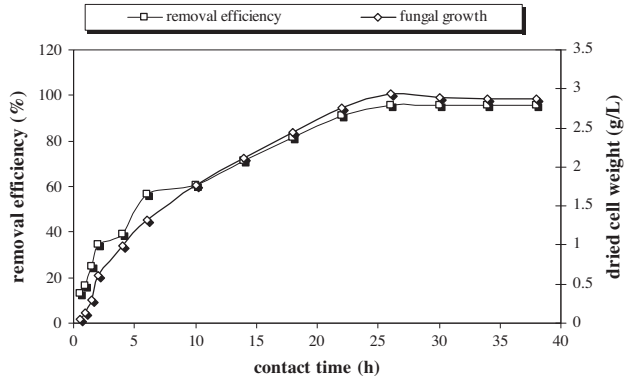

Fig. 4. Influence of contact time on fungi growth and chromium removal efficiency (T = 30˚C, initial chromium solution = 240 mg/L, agitation speed = 150 rpm).

observed, stationary growth phase was achieved after 26 h of culture and maximum biomass concentration was close to 3 g/L (Fig. 4). Chromium removal was obviously linked to growth as shown in Fig. 4 and statistically validated ($P_{value} < 0.05$). Removal efficiency increased until 26 h (98%) of culture and then remained constant; this culture time was therefore considered thereafter. The use of living microorganisms and hence the need for an acclimation time account for this phenomenon. Indeed, fungal growth led to an augmentation of the available biomass and hence an increase in sorption sites and total surface area leading to an improvement of the removal yield. Equilibrium time was higher using living microorganisms if compared to dead cells, for instance 30 min for Zn^{2+} biosorption [36] and 40 min for antimony removal from aqueous solution by lichen (*Physcia tribacia*) biomass [37]. In the removal of Cr (III) by five strains of *B. subtilis* VITSC which were isolated from Palar river basin, in the presence of chromium, stationary growth phase was observed after 22 and 28 h for *B. subtilis* strains VITSCCr01 and VITSCCr02, respectively, and after 36, 28, and 32 h for the strains VITSCCr03, VITSCCr04, and VITSCCr01, respectively [30]. Maximum sorption capacity was significantly improved by using active cells, as shown below.

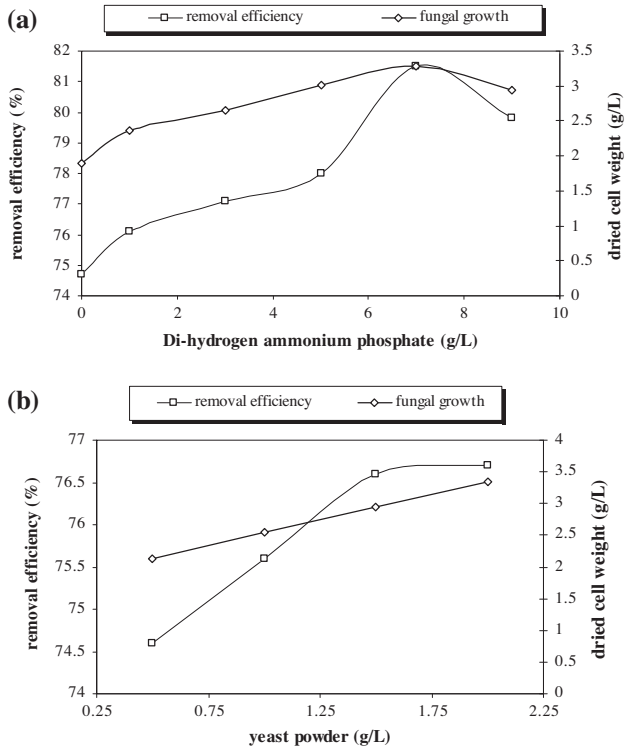

Fig. 5. Effect of nutrients addition, di-hydrogen ammonium phosphate (a) and yeast powder (b), on biomass concentration and removal efficiency (T = 30˚C, contact time = 26 h, pH=5, initial chromium solution = 240 mg/L, agitation speed = 150 rpm).

3.5. Effect of some nitrogen sources

In viable systems, adjusting the culture conditions is needed to provide essential nutrients to organism growth, since removal efficiency is related to both production of extracellular products and biomass concentration [27–29]. Since tanning effluents are deficient in nitrogen, some nitrogen sources were therefore tested for fungal growth and removal efficiency. Di-hydrogen ammonium phosphate and yeast powder were considered for this purpose. Fig. 5 shows an increase in final biomass concentrations for yeast powder and di-hydrogen ammonium phosphate additions in the range of 0.5–2 g/L and 0–7 g/L, respectively. For 2 g/L yeast powder, removal efficiency was close to 74%, while 5 g/L of ammonium phosphate were needed to achieve this removal efficiency. This showed the higher relevance of a complex substrate like yeast powder if compared with a synthetic one like ammonium phosphate. Many researchers tested various nutrients source for organism growth and pollutants removal. For the removal of Acid Navy Blue dye by *A. lentulus* FJ172995, yeast extract (10 mMN), urea (10 mMN), and ammonium chloride (10 mMN) were tested as nitrogen sources. It has been reported that maximum removal efficiency were 99.98, 99.98, and 96.37% in the presence of yeast extract, urea, and ammonium chloride, respectively, while the corresponding uptake capacities were 49.69, 98.92 and 85.11 mg/g, respectively [38]. In our previous work [31], yeast powder also showed the more significant effect on fungal growth and chromium removal (3.1 g/L biomass and 90% removal efficiency for 1 g/L yeast powder) with respect to urea and di-hydrogen ammonium phosphate.

3.6. Isotherm study

The most commonly used isotherm models are Langmuir, Freundlich and Temkin models. The Langmuir model assumes uniform energies of adsorption onto the surface and no transmigration of adsorbate in the plane of the surface. In contrast to the Freundlich equation, the Langmuir equation was developed from a theoretical standpoint to model the adsorption of gas molecules on surfaces. It was later applied to the adsorption of ions from solutions on mineral surfaces. It works reasonably well for describing ions that only bind via adsorption mechanisms. Fig. 6 shows the isotherm models plot for the present work and the related parameters are listed in Table 2. The Langmuir

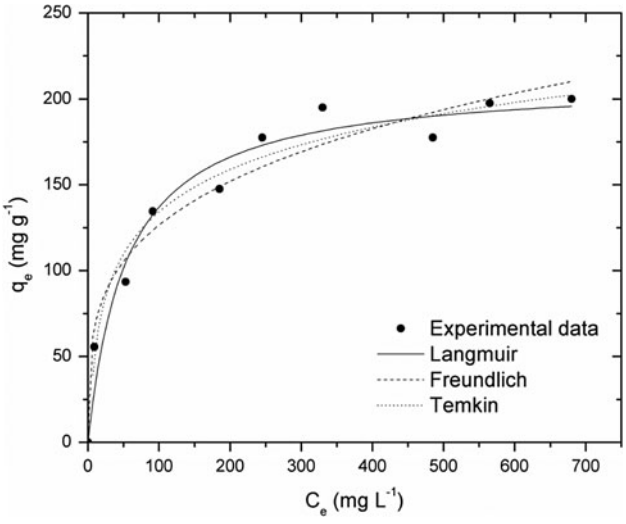

Fig. 6. Isotherm models for the present work.

Table 2
Isotherm parameters for the present work

Model	Parameters	Value	r^2
Langmuir	q_m (mg/g)	211	0.96
	b (L/mg)	0.02	
Freundlich	k_f (mg$^{1-(1/n)}$L$^{1/n}$/g)	37.37	0.96
	n	3.77	
Temkin	b_1 (kJ/mol)	68.58	0.97
	k_t (L/g)	0.44	

isotherm model was best fitted onto equilibrium data, showing a monolayer adsorption of trivalent chromium on surface of *P. chrysosporium* without interaction of the sorbed metal. However, the closeness of the regression values indicated the relevance of the three isotherm models. The surface of the adsorbent contained most likely heterogeneous moieties which were uniformly distributed on the surface, accounting for Langmuir, Freundlich, and Temkin isotherms. The maximum sorption capacity of the present medium was found to be as high as 211 mg/g according to the Langmuir isotherm constant (q_{max}), namely significantly higher than those reported in the literature, as shown in Table 3 which collected maximum sorption capacity of some biosorbent for Cr (III) sorption. The value of the Freundlich constant (n) was 3.77. By increasing the initial chromium solution from 120 to 1,080 mg/L, the separation factor (R_L) value decreased from 0.27 to 0.04. The values of Freundlich constant (n) and separation factor (R_L) demonstrated that the present system obeyed to favorable sorption process.

3.7. Kinetic study

In adsorption process, the mechanism (such as chemical reaction, diffusion control and mass transfer) was determined by the relevant kinetic model. In the present work, pseudo-first-order and pseudo-second-order models were used to determine adsorption mechanism of chromium onto the considered adsorbent. Kinetic sorption of trivalent chromium sorption

Table 3
Maximum sorption capacity by different adsorbents (viable on non-viable biosorbent) for Cr (III) uptake

Adsorbents	q_m (mg/g)	pH	Temperature (°C)	References
Waste sorghum straw	9.35	4.0	25	[3]
Waste oats straw	12.10	4.0	25	[3]
Waste agave bagasse	28.72	4.0	25	[3]
Diatomite	18.18	4.0	23	[4]
Diatomite modified with manganese chloride and sodium hydroxide	35.47	4.0	23	[4]
Diatomite modified with microemulsion	49.85	4.0	23	[4]
Kappaphycus alvarezii waste biomass	1.88	3.0	–	[7]
Spirogyra condensata	14.82	5.0	–	[8]
Rhizoclonium hieroglyphicum	12.53	4.0	–	[8]
Hylocomium splendens biomass	42.10	5.0	20	[14]
Parmelina tiliaceae biomass	52.10	5.0	20	[30]
A. oryzae	208	5.0	30	[31]
A. niger	185	5.0	30	[31]
Bentonite clay	49.75	–	–	[38]
Nannochloris oculata residual	31.70	4.8	–	[40]
P. chrysoporium	211	5.0	30	Present work

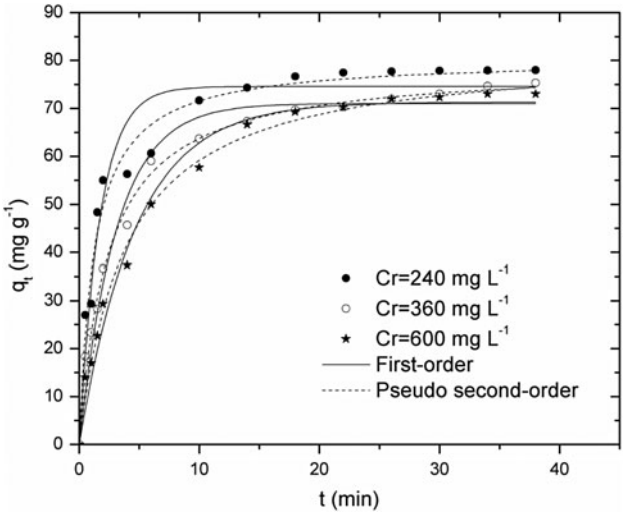

Fig. 7. Adsorption kinetic model for chromium (III) using *P. chrysosporium* biomass.

Table 4
Parameters collected from kinetic models

	Cr (III) concentration			
Kinetic model	Parameters	240 mg/L	360 mg/L	600 mg/L
Pseudo-first-order	k_1 (1/min)	0.58	0.32	0.21
	$q_{e,calc}$ (mg/g)	74.58	75.4	71.32
	$q_{e,exp}$ (mg/g)	77.96	71.36	73
	r^2	0.94	0.97	0.98
Pseudo-second-order	k_2 (g/mg min)	0.01	0.005	0.003
	$q_{e,calc}$ (mg/g)	80.43	79.06	82.01
	$q_{e,exp}$ (mg/g)	77.96	75.33	73
	r^2	0.98	0.99	0.99

by *P. chrysosporium* was determined at various initial chromium concentrations. The fitting of experimental data onto kinetic models is shown in Fig. 7 and related parameters are listed in Table 4. Linear regression analysis shows an accurate fitting of equilibrium data onto both kinetic models. However, the higher value of the correlation coefficient (r^2) shows that the pseudo-second order model was more relevant to describe trivalent chromium sorption by *P. chrysosporium* than the pseudo-first-order model. The rate constant for pseudo-first-order model increased linearly ($r^2 > 0.97$ —not shown) for increasing initial chromium concentration, in agreement with the available literature [39]. The experimental q_e values were close to those calculated.

3.8. Real effluent tests

To confirm the relevance of a given process, a validation in real conditions is needed. For this purpose, experiments have been conducted with real wastewater. The results shows that real sample composition influence drastically chromium removal and fungal growth. Chromium removal decreased to below 72%, which was lower than the value obtained for synthetic chromium solution. Fungal mass increased from 0.8 g/L to 1.9 and 2.1 g/L using di-hydrogen ammonium phosphate and yeast powder, respectively; which was lower than the values obtained with synthetic samples. Fungal mass increased with time elapse and reached stationary phase after 7.23 h. Beyond 7.23 h contact time, no fungal growth was observed. In addition, total organic carbon decreased from 30,100 mg/L to below 16,300 showing a higher need for organic carbon. The final nitrogen and phosphorous content of the effluent decreased to 11.0 and 4.3 mg/L. The observed results from real sample analysis demonstrated that *P. chrysosporium* can adapt itself to a real environment and can be efficient for the removal of trivalent chromium.

3.9. Extraction of adsorbed chromium

As mentioned previously, metals removal by viable cells is due to intracellular penetration and bounding with ligands at the surface of the biosorbent. The percentage of intracellular adsorbed chromium and external bounding one was determined. Fig. 8 shows the parentage of internal and external adsorbed chromium by the considered fungus. As can be seen, the main part of chromium was adsorbed by extracellular route. The extracellular adsorbed chromium was desorbed by 59.3, 56.0, and 57.4% in the presence of H_2SO_4, NaOH, and HNO_3, respectively. This demonstrates that H_2SO_4 was more effective than NaOH and HNO_3 for extraction of extracellular adsorbed chromium from the investigated fungus. In addition, the intracellular adsorbed chromium was desorbed by

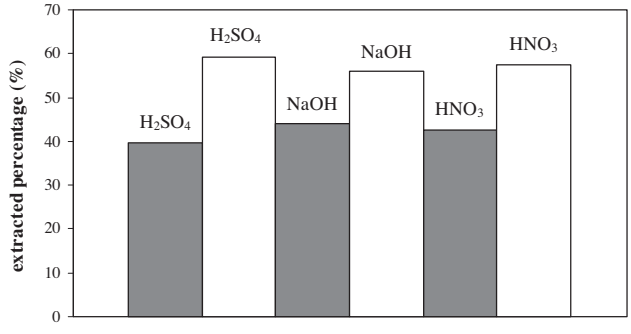

Fig. 8. Desorption of adsorbed chromium (contact time = 26 h, 150 rpm agitation speed, fungal mass = 3 g/L); intracellular (dark columns) and extracellular (open columns) adsorbed chromium.

39.7, 44.0, and 46.6% in the presence of H_2SO_4, NaOH, and HNO_3, respectively. Besides desorption percentage by various acid or alkali, these results clearly demonstrates that the main part of chromium was adsorbed by extracellular route, which is related to the bounding of chromium with exopolymeric substances such as exopolysaccharides and proteins.

4. Conclusion

In this study, the fungus *Phanerochate chrysosporium* was isolated from tanning factory environment and used for the removal of trivalent chromium. Fungal growth and chromium removal was investigated as a function of pH, temperature, contact time, nitrogen substrates addition and fungal tolerance to chromium and led to the following results:

- *P. chrysosporium* can tolerate chromium concentrations up to 600 mg/L.
- The optimal growth conditions were found to be 35˚C, pH 5 and 26 h contact time.
- A complex nitrogen source like yeast powder was more efficient than a synthetic one like di-hydrogen ammonium phosphate for fungal growth.
- Equilibrium data were best fitted onto the Langmuir isotherm model.
- Maximum sorption capacity was found to be 211 mg/g, namely significantly higher than those reported in the literature, even activated carbon.
- Experimental data was best fitted onto pseudo-second-order kinetic model with a higher value of the correlation coefficient if compared with pseudo-first-order model.
- Real sample was analyzed and demonstrated that *P. chrysosporium* can be used as an efficient biosorbent for trivalent chromium.
- Desorption test showed that the main part of chromium was bounded with extracellular compounds. In addition, the most efficient desorption was observed using H_2SO_4 for extracellular adsorbed chromium and using HNO_3 for intracellular adsorbed chromium.

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