

Biosorption of Uranium (VI) from Aqueous Solution by Pretreated *Aspergillus niger* Using Sodium Hydroxide

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ABSTRACT: The removal of uranium and any other heavy metals from wastewater might be achieved via several chemical or physical treatment techniques. Biosorption process has been considered as a potential alternative way to remove contaminants from industrial effluents. Moreover the surface of biosorbent was characterized by SEM. The biosorption characteristics of uranium (VI) on pretreated *A. niger* were evaluated as a function of pH (3.0-7.0), biomass concentration (0.05-0.4 g dry biomass/100 mL), initial uranium concentration (10-500 mg/L) and contact time (30-1440 min). The results revealed that the optimum pH was 5.0 for the adsorption of U(VI) by pretreated *A. niger*. The maximum adsorption capacity of U (VI) by pretreated *A. niger* in concentration less than 100mg/L uranium was increased significantly in comparison with live and dead biomass of *A. niger*. The metal removal was rapid with 86.4% metal sorption (43.2 mgU/g dry biomass) taking place in 30 min and the equilibrium was achieved in 240 min. The maximum uranium removal was 98.43% (16.41 mgU/g dry biomass) in concentrations 0.3g dry biomass/100mL. Adsorption process could be well defined by Langmuir isotherm with R^2 values 0.985. The kinetic data fitted through the pseudo-second-order kinetic model with the R^2 value of 0.998.

KEY WORDS: Biosorption, Uranium, Pretreated *Aspergillus niger*, Kinetic model.

INTRODUCTION

The removal of radionuclides, including uranium from water solutions that have been released as a result of human industrial activity (mining industry, conversion facilities and enrichment factories, etc.), is a practical problem [1]. Sorption could be effective to solve environmental issues on purification of water solutions from radionuclides [2, 3]. Uranium disposed can reach the top of the food chain and be ingested by humans

causing kidney or liver damage to them [4, 5]. The removal of U and any other heavy metals from wastewater can be achieved via several chemical (neutralization/precipitation) or physical (evaporation, ion exchange, membrane technology) treatment techniques. These common technologies not only require high capital investment and running costs, but also they are ineffective in low strength wastewaters. Hence, environmental engineers and

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scientists have been trying to find easy, effective, economical, and eco-friendly techniques for removal of heavy metals from aqueous solution [6-9]. Biosorption process has been increasingly considered as a potential alternative way to remove contaminants

from industrial effluents [10-12]. There are many natural adsorbents such as marine algae, bacteria, fungi, and industrial wastes, which have been used for U removal from water solutions [13-15]. Metal ion adsorption by biomass occurs through interactions with functional groups native proteins, lipids, and carbohydrates that make up the cell wall [16]. To maximize the efficiency of the biomass, it is important to identify the information obtained from these determinations, which will be useful for future [17,18].

Chemical modification of functional group is a useful technique in characterizing the functional groups responsible for metal binding [2, 19]. Sampling and analysis of Iran's uranium-content zones showed that *Fozariums. sp*, *Alternaria. sp*, *Penicillium. sp* and *A. niger* are in the soil of these areas. We investigated the adsorption of U(VI) by *A. niger*, because not only the quantity of *A. niger* was higher than that of the other fungi mentioned above, but also it can be cultivated on inexpensive medium, and it is very sensitive to small amounts of metals as well [20]. The pretreated process enhances biomass surface ions, thereby may increase or decrease the biosorption of metals from aqueous solution [21]. The objective of this study was to investigate the removal of U(VI) from aqueous solution by pretreated *A. niger* utilizing the sodium hydroxide.

EXPERIMENTAL SECTION

Preparation of materials

All the materials used in these experiments were analytical grade and the fungus used for biological adsorption was "UCF – F19/A" indigenous strain of *Aspergillus niger*. This indigenous fungus isolated and collected from infected areas of "Bandarabbas" (Lon:55.94°E, Lat:27.05°N) . The fungus was amplified on solid medium, and then it was cultivated on the liquid medium in order to produce mycelium. The solid medium was potato dextrose agar (PDA) [12, 22].

First, 39g of PDA powder was solved in distilled water and the volume of the solution increased to 1 L. Then, the container was closed with cotton and aluminum foil.

Table 1: Materials used for the preparation of liquid medium.

Chemical	g/L	Producer
Glucose	20	Merck
Peptone	10	Merck
Yeast	3	Merck

These samples were placed in an autoclave for 16 minutes until the medium becomes completely sterile (at condition of 121 °C and 1.5 bars) [22, 23]. The sterile medium was transferred to disposable sterile Petri dishes (under the microbiological hood).

Under sterile conditions using sterile forceps, fungal spores were cultured on this solid medium. After 6 days, the samples grew and reached the exponential phase. In this study, Yeast Peptone Glucose (YPG) was used as the liquid medium (presented in Table 1) [22, 24].

The appropriate pH for fungal growth in the liquid medium was pH=5.5. This pH was measured using a pH meter (Model Metrohm, 713-pH meter) and adjusted with Nitric acid and NaOH 0.1 M. Then, 100 mL of YPG was transformed into the polyethylene containers (250 mL) which provided enough space for oxygen exchange. (YPG was sterilized at 121°C, 1.5 bars, and 16 min).

Under completely sterile conditions by sterile forceps, fungal spores were removed from solid medium and were inoculated to the liquid medium to grow the fungi; (The fungal mat (0.3 g) was removed and macerated aseptically in 5.0 mL sterile medium using a homogenizer). The containers were placed into shaker incubator at the temperature 25±1°C and with a speed of 120 rpm for 5 days. At this time, the protein biomass and polysaccharides reach to maximum density at the cell surface [24, 25].

After completion of fungal growth, the fungal biomass was separated from the culture medium by filtration with Whatman No. 40 filter paper. Then, the biomass was washed several times by distilled water to completely separate it from the culture medium.

In this study, sodium hydroxide was used for biomass treatment. After separation of biomass from the liquid medium and washing them with distilled water, about 50 g of wet biomass in 500 mL sodium hydroxide (0.5N) heated for 15 minutes [22, 26]. Then, the biomass separated from the solution by Whatman No. 40 filter paper and washed with distilled water to reach pH=7 [22].

Pretreated biomass placed in the oven (at 60°C, 24h). Then, the dried biomass was crushed by mortar, and as a result, a uniform powder with 100 mesh (150 microns) obtained. This powder was kept in the refrigerator at 4°C. For the preparation of U(VI) solution, the salt of uranium [UO₂(NO₃)₂.6H₂O] was used. A stock solution of 1000 mg/L was prepared. Other concentrations were prepared by diluting the original solution [27, 28].

Biosorption studies

Four factors, including initial concentration of U(VI), pH, contact time and concentration of biomass were studied. Sampling was done twice for each point. Each sample was tested three times and the average of results was reported. After every test, the biological adsorbent was separated by filtration on Whatman No. 42 filter paper. In order to prevent sedimentation in solution, nitric acid (65%) was added. In this study, in order to determine the residual concentration of metal, inductive coupled plasma spectrometry (ICP-OES, Model GB 201A U detection limit 0.2 mg/L) was used. Scanning electron microscope (SEM) was used to study the morphological properties of the biosorbent before and after adsorption of U(VI) (WEGA TESCAN-HiVac). The following equation was used to determine the amount of U adsorbed onto the unit amount of the biomass:

$$q = \frac{V \times (C_0 - C_f)}{w} \quad (1)$$

Which;

C₀: initial concentration of metal (mg/L)

C_f: final concentration of metal (mg/L)

V: Volume of solution (L)

w: dry biomass (g)

q: amount of U adsorbed onto the unit amount of the biomass (mgU/g dry biomass). In order to evaluate metal sorption, the removal percentage of metal (R %) was also calculated. [29]

$$\%R = \frac{(C_0 - C_f)}{C_0} * 100 \quad (2)$$

Varied factors to study of U biosorption

The standard environmental constant factors were: pH =5.0, temperature 25 ± 1°C, initial concentration of U(VI) 50 mg/L, the volume of solution 100 mL, shaker

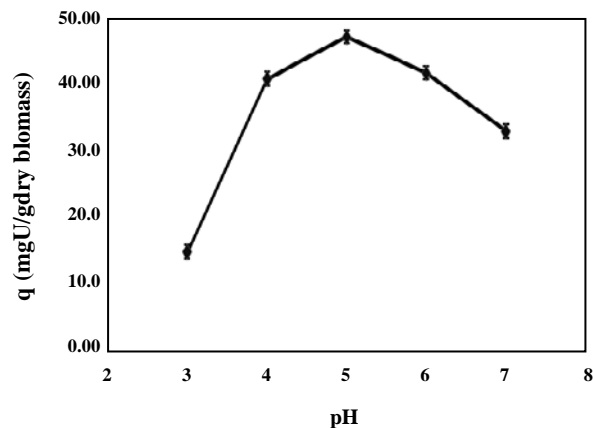


Fig. 1: The Effect of pH on U(VI) adsorption by pretreated *A. niger*.

speed 120 rpm and contact times were 2 hr. The following factors were varied, one at a time.

Five different pH levels: 3, 4, 5, 6 and 7 were studied [30,31].

Contact times of 30, 60, 120, 240, 360, 480, 1080 and 1440 min were investigated.

The concentrations of U 10, 50, 100, 200, 300 and 500 mg /L were investigated.

Five different concentrations of biomass 0.05, 0.1, 0.2, 0.3, 0.4 g dry biomass/100mL were utilized.

RESULTS AND DISCUSSION

The effect of pH on U biosorption

Earlier studies on heavy metal biosorption have shown that pH is an important parameter affecting the biosorption process. It can influence the precipitation of metal ions and ionization of surface functional groups [32]. The effect of pH on the adsorption of U(VI) onto the pretreated *A. niger* was carried out over the pH range 3.0-7.0. It was studied in order to find out the optimum pH for the biosorption process and to find out whether the biomass was able to show a good U(VI) adsorption at extreme pH values.

As shown in Fig. 1, the biomass sorption capacity increased as the pH increased from 3.0 to 5.0 and reached a maximum at a pH of 5.0. The low sorption at lower pH values could be attributed to H⁺ competition with U binding sites [33].

The adsorption rate decreased as pH rises from 5.0 to 7.0, which could be explained by the increase in the dissolved carbonate concentration with increased pH. As dissolved carbonate and bicarbonate anions increased,

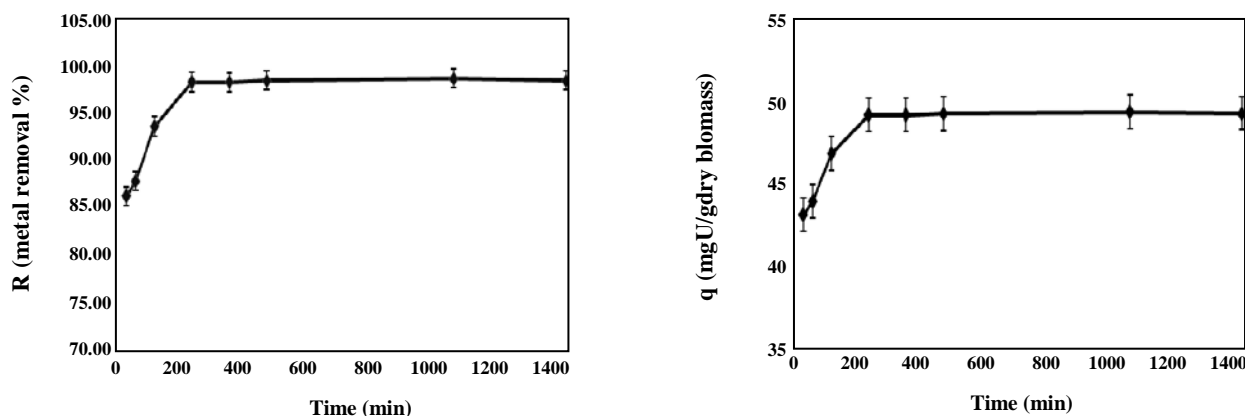


Fig. 2: The effect of contact time on U(VI) removal (a) and U(VI) adsorption (b).

they could compete adsorption sites with U(VI), thus resulting in a decrease in the adsorption amount continuously [34].

As could be seen, the significant point was that the rate of biosorption was high and almost all the U(VI) ions will be absorbed by pretreated *A. niger* around pH 5.0. Metal removal by biomass pretreated was about 95.29%, which showed that the significant amounts of U metal ions have been adsorbed from solution.

The effect of contact time on U biosorption

The most important factor influenced the chemical and biological phenomenon is the time. Important time in the biological adsorption is the equilibrium time. At this time, almost all of the active sites of adsorbent surface were occupied by metal ions and adsorbent is not able to absorb more [35]. The equilibrium time was very significant, because at that time the maximum adsorption and efficiency will be achieved.

The results showed that the amount of U(VI) adsorbed onto the biomass increased from 43.21 to 49.25 (mgU/g dry biomass), when the contact time varied from 30 to 240 min. The biosorption process was very fast, with 86.4 % of metal adsorption taking place in 30 min (see Fig. 2). The equilibrium was achieved in 240 min. At this time, the metal adsorption was obtained about 98%. It showed that more than 49 mg of U(VI) was adsorbed by active sites of pretreatment *A. niger* and less than 1 mg/L of U(VI) has remained in the solution.

As shown in the following figure (see Fig. 2), the adsorption rate decreased after 240 min, because initially a large number of vacant surface sites may be available

for adsorption and after some time, the remaining vacant surface sites may be difficult to occupy due to repulsive forces between the solute molecules of the solid and bulk

The effect of initial U concentration on U biosorption

The effect of initial U(VI) concentration on the biosorption was shown in Fig. 4. This figure showed two phases. In the first phase (initial U(VI) concentration of 0–100 mg/L), q was increased steeply; while in the second phase, it was increased slowly. The highest value of q was 117.31 mgU/g dry biomass in $C_0 = 500$ mg/L.

At low U(VI) concentrations, saturation of biomass by U ions could not be achieved since the number of U ions was smaller than the number of binding sites present on the biomass. The increase in the concentration of U in the solution results in the increase of q . [36]. Once the binding sites present on the biomass were saturated with the U, the availability of binding sites for the U decreased. This could explain why the initial stage was fast, and slowed down as the saturation was achieved.

One of the reasons for the adsorption capacity rising with initial concentration augmentation of U(VI) metal was the increase in the concentration gradient between the solution and the adsorbent surface. With increasing the metal concentration, the possibility of integrity between metal ions and adsorbent particles increases. This can result in increased metal adsorption [37]. The maximum adsorption of pretreatment *A. niger* at concentration 100 mg/L was 80.76 mgU/g dry biomass. The adsorption capacity of U(VI) with pretreated *A. niger* in uranium concentration less than 100mg/L was increased significantly in comparison with live and dead biomass of *A. niger*. [38].

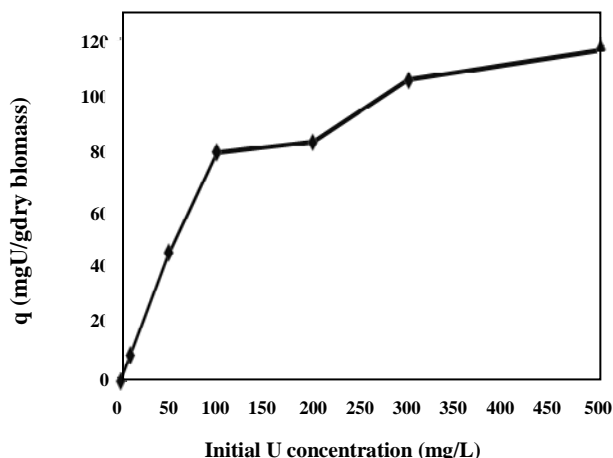


Fig. 3: q (mgU/g dry biomass) on initial U(VI) concentration.

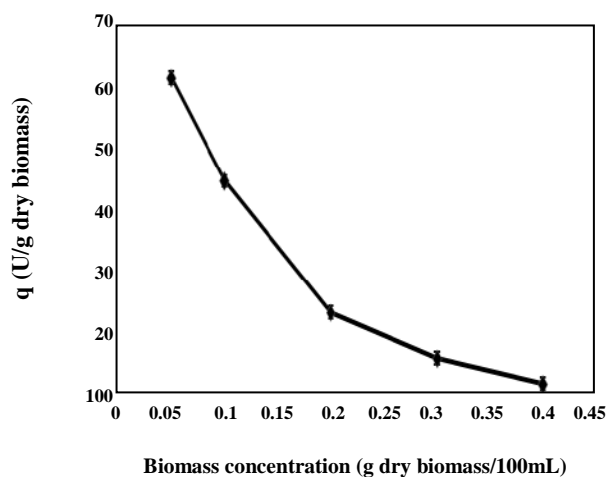
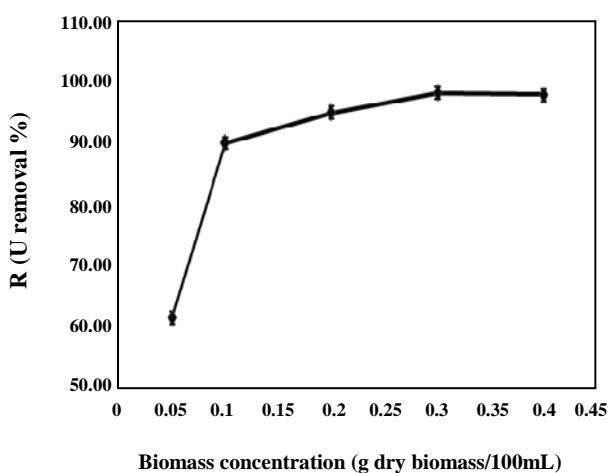


Fig. 4: The effect of Biomass concentration on U(VI) removal (a) and U(VI) adsorption (b).

This indicates that pretreated *A. niger* was efficient at low concentrations of U. This efficiency could be due to the ability of sodium hydroxide to eliminate protein and lipids that can cause the increase in the amounts of adsorbent sites such as carboxyl groups, imidazole, phosphate, sulfate, and hydroxyl that have a high capacity to bind to heavy metals in the cell wall. Moreover, it can also cause the release of polymers such as polysaccharides that have a high desire to bind with metal, when the cell walls of fungi were ruptured [13].

In industrial wastewater treatment, toxic metals at high concentrations could be separated by using chemical methods. However, the main problem was at concentrations less than 100 mg/L, because at these concentrations, the separation of metal ions by chemical was not desirable. It was worth noting that even these few concentrations are hazardous for the environment. Biological adsorbents to remove heavy metals concentrations generally less than 100 mg/L was very efficient [39] and was considered in this study.

The effect of biomass concentration on U biosorption

The effect of biomass concentration on the biosorption of U was shown in Fig. 4. As shown in Fig. 4, the maximum adsorption was achieved 98.43% at concentrations of 0.3 g dry biomass/100mL. Adsorption reduced with increasing concentrations of biomass (more than 0.3 g dry biomass/100mL). At high biomass concentration, there would have been a fast superficial adsorption onto the surface of biomass, which would have resulted in a low metal ion concentration in the solution. At a low metal ion concentration, the metal ion absorbed was low, due to the lower driving force (by a lower concentration gradient pressure). Thus, an efficient use of adsorptive capacity of the biosorbent was not reflected [36]. The remarkable thing about pretreated biomass was that with using only 0.1 g dry biomass/100mL of this adsorbent about 90 % adsorption occurred. This issue was very cost-effective.

Adsorbent characterization

Scanning Electron Microscope (SEM) was used to study the morphological properties of the biosorbent before and after treatment of biomass *A. niger* with sodium hydroxide using a microscope (VEGA/TESCAN) was shown in Fig. 5(a) at 3000×magnification

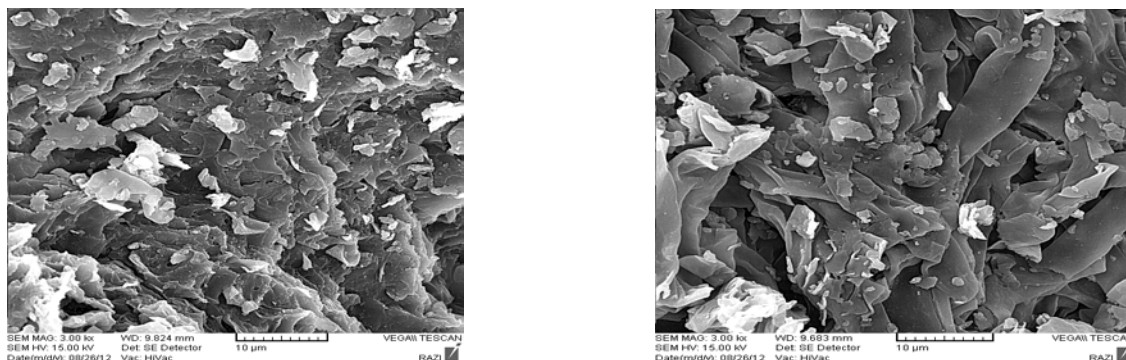


Fig. 5: SEM photos of *A. niger* before treatment of biomass (a)- SEM photos of *A. niger* after treatment of biomass (b).

(before treatment) and SEM micrographs of the *A. niger* was shown in Fig. 5(b) at 3000×magnification (after treatment). Before treatment, the biosorbent displayed a porous and bitty surface structure; while after treatment, it illustrated a regular surface structure. In particular, porosity structure is essential because it may offer more binding sites and increase contact area, which facilitates pore diffusion during the treatment process [40]. The changes in the surface morphology of biosorbent before and after treatment have been shown in Fig. 5.

In some cases available macromoles in cell wall may become denatured or become deactivate. By pretreatment, probability of connection of metal ions to these functional groups will be increased, and this action by NaOH causes denaturation of proteins on cell wall. And also this causes Hydrolysis of macromoles and deacetylation of chitin and chitosan on cell wall. This will cause increase of biosorption capacity of fungi [41,42].

Sorption isotherm analysis

The equilibrium of sorption was an important physicochemical aspect for evaluating the sorption process as a unit operation. Several isotherm equations were used for equilibrium modeling the biosorption systems. The most commonly used ones were *Langmuir* and *Freundlich* equations.

The *Langmuir* model was based on the assumption that maximum sorption occurred when a saturated monolayer of the solute molecules was present on the adsorbent surface [43]. The linearized mathematical description of this model is:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{\max}} + \frac{1}{q_{\max} b} \quad (3)$$

Where:

C_e : the equilibrium concentration of metal in solution (mg/L)

q_e : the amount of metal ions adsorbed onto the unit biomass at equilibrium time (mgU/g dry biomass)

q_{\max} : (mgU/g dry biomass) and b (L/mg) were Langmuir constants related to maximum sorption capacity and sorption energy, respectively

The Freundlich equation was an empirical relationship, whereby it is assumed that the sorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. This empirical equation represents as follows:

$$q_e = K(C_e)^{1/n} \quad (4)$$

Where K and n are the Freundlich constants, the definition of C_e and q_e are the same as in equation (3).

In order to determine how well the Freundlich model fitted the experimental data, the plot of $\log C_e$ versus $\log q_e$ was employed. The calculated values of the Langmuir and Freundlich parameters were obtained by the least squares method and were given in Table 2.

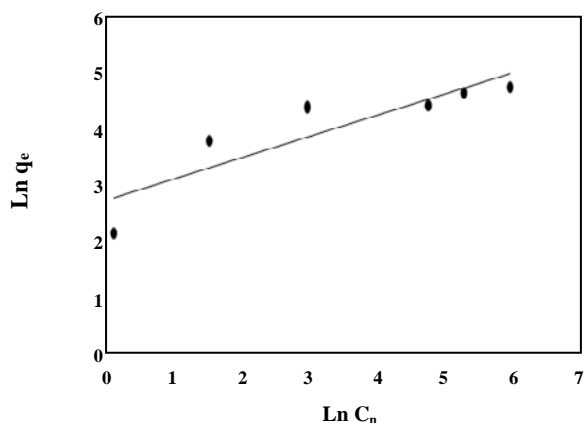
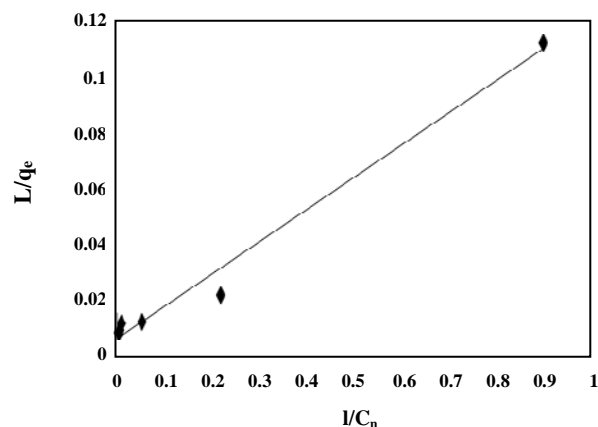
According to the Table 2, the correlation coefficients (R^2) of Langmuir and Freundlich isotherms were 0.9851 and 0.7972 respectively. The correlation coefficient of Langmuir isotherm was higher than that of Freundlich model for pretreated *A. niger*. This indicates that the Langmuir isotherm for pretreated *A. niger* was fitted better and was accepted for this adsorbent.

Kinetic modeling

Kinetics of uranium (VI) uptake was modeled using the pseudo-first-order and pseudo-second-order Lagergren equation. The pseudo-first-order reaction

Table 2: Calculated Langmuir and Freundlich coefficients.

Langmuir parameters		
q_{\max} (mgU/g dry biomass)	b (L/mg)	R^2
149.25	0.0583	0.9851
Freundlich parameters		
n	k	R^2
2.65	15.7	0.7972

Fig. 6: The Freundlich isotherm of sorption U(VI) on the pretreated *A. niger*Fig. 7: The Langmuir isotherm of sorption U(VI) on the pretreated *A. niger* concentration.

of Lagergren for sorption can be expressed as follows [44-46]:

$$dq_t/dt = k_1(q_e - q_t) \quad (5)$$

where q_e and q_t are the amount of metal adsorbed per unit weight (mg/g dry biomass) of biosorbent at equilibrium and at any time t (min) respectively and k_1 is the rate constant of pseudo-first-order sorption (min^{-1}). The integrated equation after applying the boundary conditions, for $t=0$, $q_t=0$, form of the above becomes:

$$\log(q_e - q_t) = \log(q_e) - (k_1/2.303)t \quad (6)$$

The value of the rate constant (k_1) and q_e for the pseudo-first-order sorption reaction can be obtained by plotting

$$\log(q_e - q_t) \text{ versus } t.$$

The pseudo-second-order rate of Lagergren [44] can be expressed as follows:

$$dq_t/dt = k_2(q_e - q_t)^2 \quad (7)$$

Where k_2 ($\text{g}/(\text{mg min})$) is the rate constant for the pseudo-second-order sorption. The integrated linear form of Eq. (7) can be represented as follows:

$$t/q_t = 1/(k_2q_e^2) + (1/q_e)t \quad (8)$$

The pseudo-second-order rate constant (k_2) and q_e can be calculated from the intercept and slope of the linear plot of t/q_t versus t . The plots for pseudo-first-order kinetics, $\log(q_e - q_t)$ versus t , and pseudo-second-order kinetics, t/q_t versus t were shown in Fig. 8(a) and (b), respectively. The kinetics data could be well described by pseudo-second-order plot.

The observed experimental values for q_e , and the values obtained from the plots of pseudo-first-order, and pseudo second-order kinetics were shown in Table 3. The R^2 value of pseudo-first-order rate kinetics was 0.941 and for pseudo-second order, rate kinetics was 0.998. The observed experimental q_e values were close to the values of q_e obtained from the slope of the linear plot for the pseudo-second-order rate kinetics (t/q_t vs. t), as shown in Table 3. Therefore, the pseudo-second-order rate kinetic model best described the experimental data.

The initial rate of uptake (h) was calculated using the expression $h = k_2q_e^2$. It was found to be 7.7 (mg/g) min.

CONCLUSIONS

This study has shown that the pretreated *A. niger* using sodium hydroxide can effectively remove U(VI)

from aqueous solution. The sorption performances were controlled by solution pH, contact time, initial U(VI) concentration, and adsorbent concentration. The maximum adsorption capacity of biomass was observed at pH 5.0. Biosorption of U(VI) onto pretreated *A. niger* was very rapid with more than 86.4% of metal biosorption taking place in 30 min. However, the equilibrium could be achieved in 4hr of contact time. The results showed that the maximum adsorption of U(VI) was 98.43% in concentrations 0.3g dry biomass/100mL. The results showed that with only 0.1g dry biomass/100mL, more than 90 % adsorption occurred, that was very cost-effective. The kinetic data fitted the pseudo-second-order kinetic model with the R^2 value of 0.998. Adsorption process could be well defined by Langmuir isotherms with R^2 values 0.985. Pretreated *A. niger* using sodium hydroxide was an efficient and economical method for the removal of U(VI) from industrial wastewater.

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REFERENCES

- [1] Bai J., H Yao F., Fan M., Lin L., Zhang H., [Biosorption of Uranium by Chemically Modified *Rhodotorula Glutinis*](#), *J. Environ. Radioact.*, **101**: 969-973 (2010).
- [2] Bai R.S., Abraham, T.E., [Studies on Enhancement of Cr\(VI\) Biosorption by Chemically Modified Biomass of *Rhizopus Nigricans*](#), *Water Res.*, **36**: 1224-1236 (2002).
- [3] Anke M., Seeber O. , Müller R., Schäfer U., [Transfer in the Food Chain from Soil to Plants, Animals and Man](#), *Geo chem. J.* , **69**:75-90 (2009).
- [4] Samson R.A., Houbraken J., Summerbell R.C., Flannigan B., Miller J.D., [Common and Important Species of Fungi and Actinomycetes in Indoor Environments. In: Microorganisms in Home and Indoor Work Environments](#), *Taylor and Francis Journal*, **61**: 287–292 (2001).
- [5] Dursun A., Comparative Study on Determination of the Equilibrium, Kinetic and Thermodynamic Parameters of Biosorption of Copper (II) and Lead (II) Ions Onto Pretreated, *Aspergillus niger*, *Biochem. Eng. J.*, **28**: 187-195 (2006).
- [6] Muhamad H., Doan H., Lohi A., [Batch and Continuous Fixed-Bed Column Biosorption of Cd²⁺ and Cu²⁺](#), *Chem. Eng. J.*, **158**: 369-377 (2010).
- [7] Kalantari H., Yaghmaei S., Roostaazad R., Mohammad Beigi H., [Removal of Zirconium from Aqueous Solution by *Aspergillus niger*](#), *SCI Iran J.*, **21(3)**: 772-780 (2014).
- [8] Naddafi K., Nabizadeh R., Saeedi R., Mahvi A. H., Vaezi F., Yaghmaeian K., Ghasri A., Nazmara S., [Biosorption of Lad\(II\) and Cadmium\(II\) by Protonated *Sargassum Glaucescens* Biomass in a Continuous Packed Bed Column](#), *J. Hazard. Mater.*, **147**: 785-791(2007).
- [9] Bashardoost R., Vahabzadeh F., Shokrollahzadeh S., [Sorption Performance of Live and Heat-Inactivated Loofa-Immobilized *Phanerochaete chrysosporium* in Mercury Removal from Aqueous Solution](#), *Iran. J. Chem. Chem. Eng. (IJCE)*, **29(4)**: 79-89 (2011).
- [10] Kuber C., Bhainsa S.F., D'Souza., [Removal of Copper Ions by the Filamentous Fungus, *Rhizopus Oryzae* from Aqueous Solution](#), *Bioresour. Technol.*, **99**: 3829-3835 (2008).
- [11] Khani M.H.; Keshtkar A.R.; Meysami B.; Zarea, M.F.; Jalali, R., [Biosorption of Uranium from Aqueous Solutions by Nonliving Biomass of *Marinealgae Cystoseira Indica*](#), *Electron. J. Biotechnol.*, **9**: 100-106 (2006).
- [12] Akar T., Tunali S., Kiran I., [Botrytis Cinerea as a New Fungal Biosorbent for Removal of Pb\(II\) from aqueous solutions](#), *Biochemistry Eng. J.*, **25**: 227-235(2005).
- [13] Ahluwalia S.S., Goyal D., [Microbial and Plant Derived Biomass for Removal of Heavy Metals from Wastewater](#), *Bioresour. Technol.* **98**: 2243-2257(2007).
- [14] Cabuk A., Ilhan S., Filik C., Caliskan F., [Pb²⁺ Biosorption by Pretreated Fungal Biomass](#), *Turk. J. Biol.*, **29**: 23-28(2005).
- [15] Yan G., Viraraghavan T., [Heavy Metal Removal from Aqueous Solution by Fungus *Macro Rouxii*](#) , *Water. Res.*, **37**:4486-4496 (2003).
- [16] Chen X.C., et al., [Determination of Copper Binding in *Pseudomonas Putida* CZ1 by Chemical Modifications and X-Ray Absorption Spectroscopy](#), *Appl. Microbiol. Biotechnol.*, **74**: 881-889 (2007).

- [17] Margarete Kalin W. N., Wheeler G., [The Removal of U from Mining Wastewater Using Algal/Microbial Biomass](#), *J. Environ. Radioact.*, **78**: 151-177(2004).
- [18] Nuhoglu Y., Oguz E., [Removal of Copper \(II\) from Aqueous Solutions by Biosorption on the Cone Biomass of *Thujaorientalis*](#), *Process. Biochem.*, **38**: 1627-1631 (2003).
- [19] Holan Z.R., Volesky B., [Accumulation of Cadmium, Lead, and Nickel by Fungal and Wood Biosorbents](#), *Appl. Biochem. Biotechnol.*, **53** : 133-146 (1995).
- [20] Mulder E.G., Sur l'influence de Cuivre Sur La Croissance Des Microorganismes, *Ann Ferm.n.s.*, **4** : 513-533(1938).
- [21] Mukhopadhyay M., Noronha S. B., Suraishkumar G.K., [Kinetic Modeling for the Biosorption of Copper by Pretreated *Aspergillus Niger* Biomass](#), *Bioresour. Technol.*, **98**: 1781–1787 (2007).
- [22] Tsekova K., Todorova D., Dencheva V., Ganeva S., [Biosorption of Copper \(II\) and Cadmium \(II\) from Aqueous Solutions by Free and Immobilized Biomass of *Aspergillus Niger*](#), *Bioresour. Technol.*, **101**: 1727-1731 (2010).
- [23] Dajjanutat P., Promkotras S., Kaewkan netra P., [Biosorption and Desorption of Cadmium from Contaminated Water Using Kaialgae of *Cladophora spp* as Biosorption in Biofilter](#), *Queen Siriki National Conversion Center Bangkok Thailand*, **52**: 72-80 (2009).
- [24] Jianlong W., Xinmin Z., Decai D., Ding Z., [Bio Absorption of Lead \(II\) from Aqueous Solution by Fungal Biomass of *Aspergillus. Niger.*](#), *Biotechnol. J.*, **87**: 273-277 (2001).
- [25] Ahmad I. M., Ansari I., Aqil F., [Biosorption of Cr and Cd by Metal Tolerant *Aspergillus sp* and *Penicillium sp* Using Single and Multi-Metal Solution](#), *Indian J. Exp. Biol.*, **44**(1):73-76 (2006).
- [26] Chubar N., Carvalho J.R., Correia M.J.N., [Heavy Metals Biosorption on Cork Biomass Effect of the Pretreatment](#), *Physicochem. Eng. J.*, **238**: 51-58 (2004).
- [27] Kurniawan T., Chan G., Lo W., Babel S., [Physico-Chemical Treatment Techniques for Waste Water Laden with Heavy Metals](#), *Chem. Eng. J.*, **118**: 83-98 (2006).
- [28] Narsi R., Bishnoi G., [Fungus-an Alternative for Bioremediation of Heavy Metal Containing Wastewater](#), A review, *J. Sci. Ind. Res.*, **64**: 93-100 (2005).
- [29] Dang V. H., Doan H. D., Dang T., Lohi A., [Equilibrium and Kinetics of Biosorption of Cadmium \(II\) and Copper \(II\) Ions by Wheat Straw](#), *Bioresour. Technol.*, **54**: 102-112 (2008).
- [30] Li X., Liao D., Xu X., Yang Q., Zerg G., Zheng W., Guo L., [Kinetic Studies for the Biosorption of Lead and Copper Ions by *Penicillium Sinplicissimum* Immobilized Biomass](#), *J. Hazard. Mater.*, **73**: 234-241 (2008).
- [31] Çabuk A., Akar T., Tunali S., Gedikli S., [Biosorption of Pb\(II\) by Industrial Strain *Saccharomyces Cerevisiae* Immobilized on the Biomatrix of Cone Biomass of *Pinus Nigra*: Equilibrium and Mechanism Analysis](#), *Chem. Eng. J.*, **131**: 293–300(2007).
- [32] Gok C., Aytas S., [Biosorption of U \(VI\) from Aqueous Solution Using Calcium Alginate Beads](#), *J. Hazard. Mate.*, **168**: 369-375 (2009).
- [33] Parab H., Joshi S., Shenoy N., Verma R., Lali A., Sudersanan M., [U Removal from Aqueous Solution by Coir Pith: Equilibrium and Kinetic Studies](#), *Bioresour. Technol.*, **96**: 1241-1248 (2005).
- [34] Barnett M.O., Jardine P.M., Brooks S.C., Selim H.M., [Adsorption and Transport of U\(VI\) in Subsurface Media](#), *Soil Sci. Soc. Am. J.*, **64**: 908-917 (2002).
- [35] Khani M.H., Keshtkar A.R., Ghannadi M., Pahlavanzadeh H., [Equilibrium Kinetic and Thermodynamic Study of the Biosorption of U Onto *Cystoseria Indica* Algae](#), *J. Hazard. Mater.*, **150**: 612-618 (2008).
- [36] Saxena S., Prasad M., D'Souza S.F., [Radiionuclide Sorption Onto Low-Cost Mineral Adsorbent](#), *Ind. Eng. Chem. Res.*, **45**: 9122-9128 (2006).
- [37] Vikas S., Bhat a.b., Melo J.S., Chaugule B.B., D'Souza S.F., [Biosorption Characteristics of U \(VI\) from Aqueous Medium Onto *Catenella Repens* a Red Alga](#), *J. Hazard. Mate.*, **158**: 628-635 (2008).
- [38] Sana S., Roostaazad R., Yaghmaei S., [Biosorption of Uranium from Aqueous Solution by Live and Dead *Aspergillus Niger*](#), *J. Hazard. Toxic Radioact. Waste.*, **18**(3),(july2014).

- [39] Lebeau T., Bagot D., Jézéquel K., Fabre B., [Cadmium Biosorption by Free and Immobilised Microorganisms Cultivated in a Liquid Soil Extract Medium: Effects of Cd, pH and Techniques of Culture](#), *Sci. Total. Environ.*, **291**: 73-83 (2002).
- [40] Cui Panga b.c., Yun.Hai L. A., Xiao Hong C., Min L., Gou Lin H., Rong H., [Biosorption of U\(VI\) from Aqueous Solution by Dead Fungal Biomass of *Penicillium Citrinum*](#), *Chem. Eng. J.*, **170**: 1-6 (2011).
- [41] Ulay G., Glu B., Elik G.C., Arica M., [Studies on Accumulation of Uranium by Fungus *Lentinus Sajor-Caju*](#), *Journal of Hazardous Materials*, **136** : 345–353 (2006).
- [42] Xie S., Yang J., Chen C., Zhang X., Wang Q., Zhang C., [Study on Biosorption Kinetics and Thermodynamics of Uranium by *Citrobacter Freudii*](#), *Journal of Environmental*, **21**: 296-302 (2008).
- [43] Volesky B., [Biosorption Process Simulation Tools](#), *Original Research Article Hydrometallurgy*, **71**: 179-190 (2003).
- [44] Ho Y.S., McKay G., [Pseudo-Second Order Model for Sorption Processes](#), *Process Biochem.*, **34**: 451-46 (1999).
- [45] Bhat S. V., Melo J.S., Chaugule B.B., D'Souza S.F., [Biosorption Characteristics of Uranium\(VI\) from Aqueous Medium onto *Catenella Repens* a Red Alga](#), *J.Hazard. Mate.*, **158**:628-635 (2008).
- [46] Wang J.S., Hu X.J., Liu Y.G., Xie S.B., Bao Z.L., [Biosorption of Uranium \(VI\) by Immobilized *Aspergillus Fumigatus* Beads](#), *Chem. Eng. J.*, **170**: 1–6 (2011).