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Mycoremediation of cadmium from aqueous solution using newly isolated *Actinomucor* sp.: isotherm and kinetic studies

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ABSTRACT

The fungus M2904 was isolated from farmland soil samples with heavy metals contamination. Our primary evaluation suggested that the isolate M2904 shows high tolerability and growth in exposure to six heavy metals in batch culture. The minimum inhibitory concentration (mg L^{-1}) for M2904 was 2,000 (Cu^{2+}, Zn^{2+} and Ni^{2+}), 1,000 (Pb^{2+}), 1,500 (Hg^{2+}) and 4,000 (Cd^{2+}). The molecular identification revealed that the M2904 belongs to the genus *Actinomucor* with the identity of 89% in internal transcribed spacer gene region. Functional groups in the process of mycoremediation were determined using Fourier transform infrared analysis, and results showed that phosphate, carboxyl, amine and hydroxyl groups connected to the cadmium ions in the surface of biosorbents. These results were verified by scanning electron microscopy analysis. The isotherm models indicated that *Actinomucor* sp. cadmium adsorption follows the Langmuir model. The maximum capacity (q_{max}) for cadmium adsorption for the dry biomass was determined 24.03 mg g^{-1}. The optimum temperature, initial concentration, contact time and pH for cadmium biosorption by the absorbent was measured 45°C, 400 mg L^{-1}, 120 min and 7–8, respectively. Pretreatment of adsorbent by 0.5 N NaOH significantly increased cadmium adsorption capacity. Based on correlation coefficient and experimental adsorption capacity, adsorption kinetics of *Actinomucor* sp. follow the pseudo-second-order equation (R^2 = 0.9996). Biosorbent recovery showed that 87% of the adsorbed cadmium can be separated from the fungal biomass by 0.1 M nitric acid (HNO_3) treatment after 2 h. The overall results show that *Actinomucor* sp. is a strong biosorbent for removal of cadmium ions from contaminated wastewaters.

Keywords: Actinomucor sp.; Biosorption; Cadmium; Heavy metal; Mycoremediation

1. Introduction

Heavy metals are highly hazardous pollutants and their release to the environment is a matter of concern all over the world due to non-degradability, high toxicity and their accumulative features [1]. Therefore, removal of these contaminants is essential from the standpoint of public health and environmental pollution control agencies [2].

Cadmium is a toxic and carcinogenic heavy metal widely scattered in the environment. The main cadmium pollution sources are plating, alloy, battery industries and agricultural wastes [3]. Enhanced cadmium concentration leads to the increase in related diseases such as kidney disorders, bone diseases, cancer and chromosomal effects, also kidney tubule damages are caused by long-term exposure to low concentrations [4]. The methods for removing heavy metals are divided into three general categories including physical, chemical and biological [5]. Since the physical and chemical methods are expensive and only applicable to the high concentration of heavy metals,

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in recent years biological techniques have been highly regarded [6]. Yeast and fungi are unique in heavy metal adsorption features. In most fungi, chitin $(C_8H_{13}O_5N)_n$ is the main material, studies have revealed that chitin and its derivatives including bioadsorbent groups can be used for adsorbing heavy metals [7]. In these studies, the fungi that produce huge amounts of cell biomass are introduced as the powerful adsorbents of various metals.

In the current study, mycoremediation of Cd^{2+} by dry biomass of *Actinomucor* sp. in aqueous solution was examined. The biosorption capacity, heavy metal tolerance, Fourier transform infrared (FTIR) analysis and the kinetics and isotherm studies were assessed to gain insight into the role of functional groups and biosorption mechanism that are connected to cadmium ions in this fungal strain. Therefore, the innovative aspect of this study was to introduce a new fungal strain as an efficient cadmium biosorbent and review the mechanism of its adsorption.

2. Materials and methods

2.1. Sample collection and isolation of cadmium resistant fungi strains

In total, 29 soil samples were collected from heavy metals contaminated areas such as farmlands, mines adjacent areas and oil contaminated locations. Cadmium nitrate tetrahydrate ($Cd(NO_3)_2.4H_2O$) (Merck, Germany) was used for making the cadmium standard solution. For primary isolation of the cadmium resistant fungi, the spread-plate method was used on the potato dextrose agar (PDA) media, containing Cd^{2+} solution at a concentration of 300 and 50 mg L^{-1} tetracycline (to prevent the growth of bacteria). 0.1 g of each sample was weighed and added to 10 mL sterile normal saline. In addition, serial dilution was prepared by adding 1 mL of each solution to 9 mL sterile normal saline. The final diluted solutions vortexed for 15 s. Eventually, 100 μL of each final dilution was cultured on the PDA medium containing cadmium and antibiotics and evenly distributed using sterile L-shaped glass tube. The cultured plates were incubated at 28°C for 2 weeks. Filamentous fungi colonies were purified and culture maintenance on PDA medium at 4°C. Primary identification of the different pure cultures was carried out based on micromorphology and colony features [8].

2.2. Evaluation of tolerance index and cadmium biosorption analysis

In order to evaluate the tolerability of isolates obtained from the initial screening stage, each isolate was inoculated into the potato dextrose broth (PDB) medium under liquid condition containing 2,000 mg L^{-1} cadmium [9]. For inoculation, 1 cm^2 of fresh culture PDA medium was cut and inoculated into the PDB medium. The flasks were shaken at 170 rpm and at 28°C on a rotary shaker at 170 rpm for a week. Fungal growth on PDB medium was indicated as a resistance strains. Regarding to cellular growth rate in the previous step, five strains were selected for the study of cadmium mycoremediation. Accordingly, selected isolates were incubated in PDB medium (pH 6) with the cadmium concentration of 500 mg L^{-1} at 28°C on a rotary shaker at 170 rpm for a week, followed by batch culture and centrifugation. Then, the supernatant was examined to determine the amount of Cd^{2+} using inductively coupled plasma (ICP) (Shimadzu ICP-7500, Japan) [10]. Each treatment replicated three times.

2.3. Determination of the tolerability against other metals

So as to investigate the effect of different ions on the growth of selected isolates, the tolerance test was done on five strains for a variety of heavy metal ions of Hg^{2+}, Cu^{2+}, Pb^{2+}, Ni^{+} and Zn^{2+}. For this purpose, under liquid and solid cultivation the minimum inhibitory concentrations (MICs) were determined by examining the fungal growth on PDA and PDB medium. Different concentrations of each metal from 500 to 4,000 mg L^{-1} were prepared. The pH of the medium adjusted at 6 and samples were incubated at 28°C for a week at 170 rpm. Turbidity was indicated as a positive fungal growth. For solid cultivation, each metal and control plate was cultivated into the different PDA medium. Fungi mycelia obtained from liquid culture were cultured on plates in triplicate. Changes in colony diameter on the spotted position after incubation at 28°C for 7 d were then studied. The MIC was defined as the heavy metal MIC that obviously stopped the fungal growth in the liquid and solid medium. Each treatment replicated three times [11].

2.4. Molecular identification of filamentous fungi

To identify the M2904 isolate, molecular identification methods were applied. The biomass was first harvested from the fungal culture in PDB medium using the shake flask method. After inoculation, the flasks were incubated at 28°C and 170 rpm for 3 d, and the biomass was then washed with distilled water. Polymerase chain reaction (PCR) based molecular identification was performed using the DNA isolated by the phenol–chloroform method, and the internal transcribed spacer (ITS) region primers including ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) synthesized by Bioneer Corporation, South Korea. Amplification was performed with a SensoQuest™ thermal cycler. The PCR mixture consisted of 12.5 μL of Taq DNA Polymerase Master Mix Red (2×) Amplicon™, 0.1 mM of each primer and approximately 200 ng of extracted DNA as a template in a total volume of 25 μL. The PCR program was as follows: 95°C for 5 min, followed by 30 cycles of 96°C for 30 s, 55°C for 90 s and 72°C for 30 s. Finally, there was an extension step at 72°C for 10 min. The PCR products were analyzed by electrophoresis on 1.0% (w/v) agarose gel, and purification was done for sequencing. A neighbor-joining phylogenetic tree for obtained sequence was constructed using the BioEdit and Molecular Evolutionary Genetics Analysis package (MEGA version 4) softwares.

2.5. Isotherm studies

Cd^{2+} adsorption isotherm test was done on the dry biomass of M2904 isolate. Different concentrations of 50, 100, 200, 400 and 800 mg L^{-1} of cadmium ion and the amount of 5 g L^{-1} of the adsorbent were prepared. The distribution of the adsorbent species among liquid and biosorbent was

described mathematically. The following models are biosorption isotherms. These isotherms explain the relationship between metal uptake per unit weight of biosorbent (q_e) and the equilibrium adsorbent concentration (C_e) such as Langmuir and Freundlich isotherms. The biosorption equilibrium isotherm was obtained by following models and their equations. The Langmuir model has been applied for the assessment of maximum metal adsorption at various initial metal concentrations assuming that the adsorption occurs at specific homogeneous parts, while maximum biosorption takes place on the biosorbent surface consisted of a saturated solute monolayer, with the constant energy of biosorption and no biosorbate molecules migration in the surface site, represented by the following equations [12]:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}} \tag{1}$$

$$R_L = \frac{1}{1 + bC_0} \tag{2}$$

where q_e (mg g^{-1}) is the amount of metal ions adsorbed per unit mass of adsorbent, C_e is the equilibrium concentration in the solution (mg L^{-1}), $q_{\max}$ is the maximum adsorption capacity (mg g^{-1}), b denotes the Langmuir constant (L mg^{-1}), C_0 (mg L^{-1}) is the initial concentration of Cd^{2+} and R_L is the constant separation factor [13].

Freundlich isotherm model describes several biosorption energies at different sites and biosorption of solutes from a liquid to a solid surface. This model is an empirical equation based on an exponential distribution of sorption sites and energies, given by the following equation where K_f and n are the Freundlich constants and are indicators of adsorption capacity and adsorption intensity, respectively. q_e is the metal adsorbed amount per unit weight of biosorbent (mg g^{-1}), C_e is the equilibrium metal concentration (mg L^{-1}) [14]:

$$\log q_e = \log K_f + \left(\frac{1}{n}\right) \log C_e \tag{3}$$

These models can provide some information about metal uptake capacity and differences among various species.

2.6. Kinetic studies

In order to better understand the process of metal adsorption and time equilibrium, kinetic models were investigated. The kinetics explains the solute adsorption, which consequently controls the adsorbent residence time at the solution–solid interface. Adsorption kinetics was studied after 2, 5, 15, 30, 60, 120 and 240 min at the concentration of 200 mg L^{-1} of cadmium and 5 g L^{-1} of the adsorbent. Several models have been applied to examine experimental biosorption data. Pseudo-first-order and pseudo-second-order models have often been used to describe biosorption kinetic data. The linear expression of pseudo-first-order rate based on biosorption capacity is generally described by the following equation [15]:

$$\ln\left(q_e - q_t\right) = \ln q_e - K_1 t \tag{4}$$

where q_e and q_t are the biosorbed amount of metal per unit weight (mg g^{-1} dry weight) of adsorbent at equilibrium and at any time t (min) respectively, and K_1 is the constant rate of pseudo-first-order adsorption (min).

The pseudo-second-order equation, based on the assumption that the rate-controlling step usually correlates the behavior over the whole range of adsorption. The kinetic rate equation is expressed as [16]:

$$\frac{t}{qt} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \tag{5}$$

where q_t is the sorbate amount on sorbent at time t (mg g^{-1}), K_2 is the equilibrium constant rate of pseudo-second-order sorption kinetics (g mg^{-1} min^{-1}) and q_e is the equilibrium adsorption (mg g^{-1}). The constants were both determined by plotting $\log(q_e - q_t)$ against t and t/q_t against t, respectively.

2.7. Effect of environmental parameters

In order to determine optimal condition of biosorption, effects of pH (range of 2–8), contact time (2, 5, 15, 30, 60, 120 and 240 min), temperature (range of 15°C–55°C) and initial Cd^{2+} concentration (50, 100, 200, 400 and 800 mg L^{-1}) were investigated. Adsorption experiments were performed in the concentration of 200 mg L^{-1} Cd^{2+}, shaker at a constant speed of 170 rpm and 5 g L^{-1} the amount of used absorbent [9,11].

2.8. Pretreatment of adsorbent

Physical and chemical pretreatment methods showed enhancement or reduction in metal bioadsorption. Dry biomass of *Actinomucor* sp. was pretreated by NaOH, HNO_3, dimethyl sulfoxide (DMSO) and autoclave [7]. The biomass after each pretreatment was washed with deionized water, and then dried at 60°C for 24 h in a drying oven. Different pretreatments and concentrations were summarized in Table 1.

2.9. Fourier transform infrared spectroscopy analysis

To investigate the functional groups in the process of cadmium remediation by *Actinomucor* sp., the FTIR spectroscopy was applied. According to this, the dry biomass with and without metal in KBr pellet was used to obtain the FTIR spectra (Bruker, Germany). The spectra were at the ranges of 400–4,000 cm^{-1} [17].

Table 1
Different physicochemical pretreatment of *Actinomucor* sp. biomass

Pretreatment type	Pretreatment method	Time	References
Autoclave	121°C	15 min	[32]
0.5 N NaOH	Incubation in shaker (170 rpm, 28°C)	24 h	[36]
65% HNO_3	Incubation in shaker (170 rpm, 28°C)	2 h	[32]
50% DMSO	Boiling	15 min	[32]

2.10. SEM analysis

In order to investigate the morphology and structure of adsorbent, images from the biomass of *Actinomucor* sp. before and after biosorption of cadmium were placed on a glass slide with a double-sided carbon tape, and were coated with thin layer of gold. The biomass samples were observed using scanning electron microscopy (SEM; Zeiss, Germany). The voltage was constant at 25 kV and the microprobe was focused at 5,000× magnification.

2.11. Metal and biosorbent recovery after the biosorption process

Recovery of metal and biosorbent after the adsorption process was carried out using nitric acid as a desorbant agent in a batch system. For this assay, 0.05 g of the dry biosorbent was separated from the cadmium at the concentration of 200 mg L^{-1} by centrifugation (4,000*g*, 10 min) and concentrations of the Cd^{2+} released into the supernatant solution was determined. The treated biomass was washed with distilled water and then it was solved in 20 mL of the 0.1 M nitric acid solution and shaken for 2 h at 170 rpm. Next, the supernatant was measured to determine the cadmium desorption percentage from *Actinomucor* sp. biomass [18].

3. Results

3.1. Isolation of cadmium resistant fungal strains

At the fungal isolation step, 200 strains were isolated from 29 contaminated soil samples on PDA plate. Cadmium tolerability test for these isolates in PDB containing 2,000 mg L^{-1} led to the isolation of 59 strains. After analysis of cellular growth data in selected isolates at second step (results not provided), finally, five isolates with the highest growth rate were selected to examine the cadmium adsorption by the ICP. Our results at the concentration of 500 mg L^{-1} showed that the isolate M2904, with about 45% of cadmium adsorption is the most potent adsorbent strain among the other selected isolates (Fig. 1).

3.2. Determination of minimum inhibitory concentration for other metals

The MIC values of M2904 to the six different heavy metals are presented in Table 2. Our results showed that the isolate M2904 is able to grow at concentrations of 2,000 mg L^{-1} Cu^{2+}, Ni^{+} and Zn^{2+} metal ions (Table 2). The MIC result for Hg^{2+} and Pb^{2+} was 1,000 and 1,500 mg L^{-1}, respectively (Table 2). M2904 showed the highest MIC for cadmium at 4,000 mg L^{-1}. Our results showed that M2904 has high tolerance for common hazardous heavy metals, especially for cadmium.

3.3. Identification of the superior isolate

ITS gene alignment for the isolate M2904 and the alignment with the GenBank database showed 89% identity closely related to the genus *Actinomucor*. Considering to this similarity, we classified M2904 into the genus *Actinomucor* sp. The sequence was registered at the database of NCBI GenBank under the accession numbers of KT935266, and was also coded at the University of Tehran Microbial Collections (UTMC) under the access code of 5048. Fig. 2 shows the phylogenetic tree of the *Actinomucor* sp. UTMC 5048 using ITS primers and the neighbor-joining algorithm.

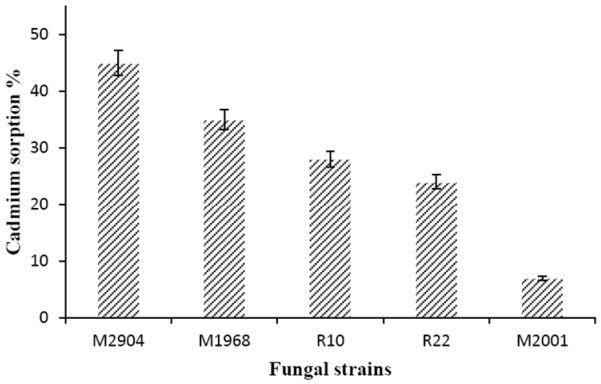

Fig. 1. The results of Cd^{2+} biosorption (%) from superior fungal strains.

Table 2
Minimum inhibitory concentration (MIC) of different heavy metals against *Actinomucor* sp.

	Actinomucor sp.
Hg^{2+}	1,500
Cu^{2+}	2,000
Pb^{2+}	1,000
Ni^{2+}	2,000
Zn^{2+}	2,000
Cd^{2+}	4,000

Note: Metal concentration (mg L^{-1}).

3.4. Isotherm study

To evaluate the cadmium adsorption process by the selected isolate, adsorption isotherm models were applied. The maximum adsorption capacity was determined 24.03 mg g^{-1}, based on the Langmuir model for the *Actinomucor* sp. (Table 2). The results of isotherms on dry biomass of *Actinomucor* sp. also showed that cadmium adsorption process of this strain follows the Langmuir model. Different Freundlich and Langmuir isotherm parameters for the strain *Actinomucor* sp. is presented in Table 3. Langmuir and Freundlich isotherm diagram for the strain *Actinomucor* sp. is presented in Fig. 3. The results showed that the adsorption model for *Actinomucor* sp. follows the Langmuir model with a correlation coefficient: 0.99.

3.5. Kinetic study

The kinetics in a mycoremediation process provides a valuable insight into the reaction pathways and the mechanism of the sorption reaction. Two common models, pseudo-first-order and pseudo-second-order, have been widely used to predict the best biosorption and parameters kinetics. Based on correlation coefficient and experimental adsorption capacity, the adsorption kinetics of *Actinomucor* sp. follow the pseudo-second-order equation. Pseudo-first-order and pseudo-second-order model

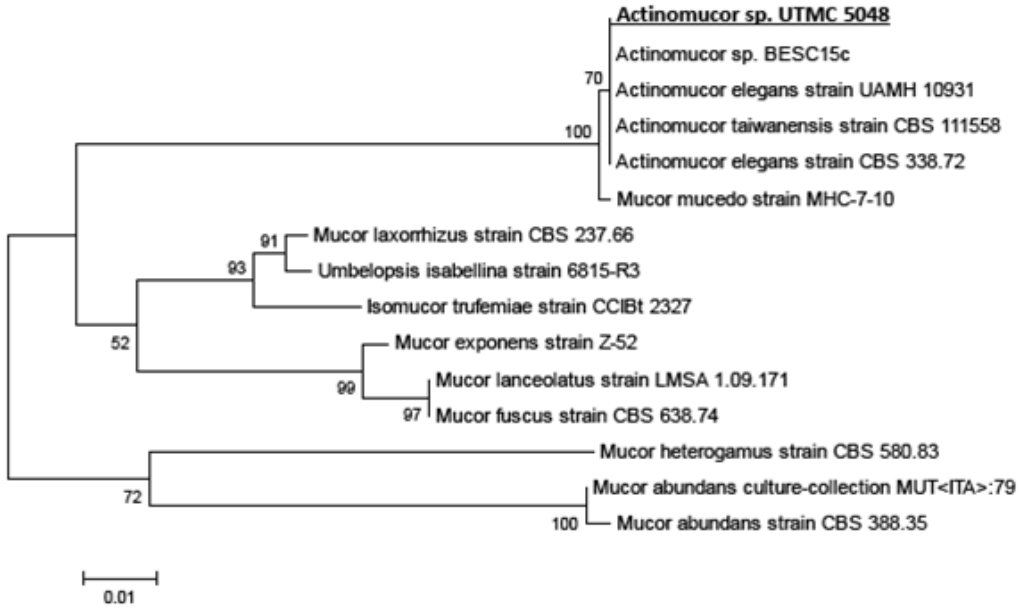

Fig. 2. The phylogenetic tree for the *Actinomucor* sp. using Mega 5 software.

Table 3
Different Freundlich and Langmuir isotherm parameters for the strain *Actinomucor* sp.

Isotherm models	Langmuir				Freundlich		
Constant	R^2	b (L mg^{-1})	q_{max} (mg g^{-1})	R_L	R^2	K_f (L g^{-1})	n
Amounts	0.99	0.02	24.03	0.5–0.05	0.9158	5.49	0.29

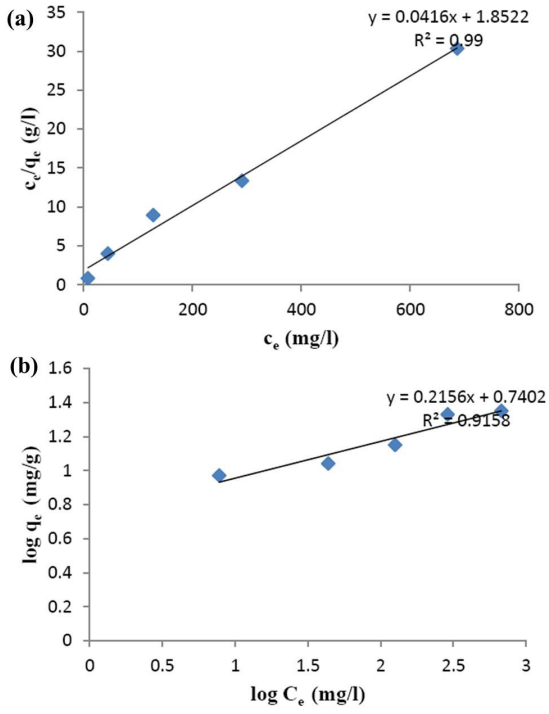

Fig. 3. Langmuir (a) and Freundlich (b) plots for the adsorption of Cd^{2+} onto dry biomass of *Actinomucor* sp.

parameters for the strain *Actinomucor* sp. are presented in Table 4. Pseudo-first-order and pseudo-second-order model diagrams for this strain are presented in Fig. 4.

3.6. Effect of environmental parameters

Cadmium removal increased with increasing pH values, adsorption capacity of the adsorbent enhanced from 7.3 to 14.2 mg g^{-1} by increasing of pH from 2 to 8 (Fig. 5(a)). The increase of pH more than 8 will be led to formation cadmium hydroxide and in result sedimentation of the cadmium ions [19]. As the temperature of the adsorption medium increased from 15°C to 55°C, the adsorption capacities of adsorbent enhanced about 10% (Fig. 5(b)). The biosorption process of Cd^{2+} onto the *Actinomucor* sp. biomass was very fast in the first 30 min and adsorption equilibrium time was obtained within 120 min (Fig. 5(c)). Batch adsorption experiment indicated that increasing the initial cadmium concentration from 50 to 800 mg L^{-1} enhanced absorption capacity from 9.44 to 22.65 mg g^{-1}, saturation range of adsorbent was determined about 400 mg L^{-1} (Fig. 5(d)). The results of this study indicated that the optimum temperature, initial concentration, contact time and pH for cadmium biosorption by the absorbent was about 45°C, 400 mg L^{-1}, 120 min and 7–8, respectively.

3.7. Pretreatment of adsorbent

Pretreatment of adsorbent by NaOH increased Cd^{2+} adsorption capacity up to 47%. However, using DMSO and

Table 4
Different pseudo-first-order and pseudo-second-order kinetic models parameters for the strain *Actinomucor* sp.

Kinetic models	Pseudo-second-order			Pseudo-first-order			q_e (exp) (mg g^{-1})
Constant	K_2 (g mg min^{-1})	q_e (cal) (mg g^{-1})	R^2	K_1 (min^{-1})	q_e (cal) (mg g^{-1})	R^2	
Amounts	0.02	13.73	0.9996	0.01	2.23	0.9194	13.5

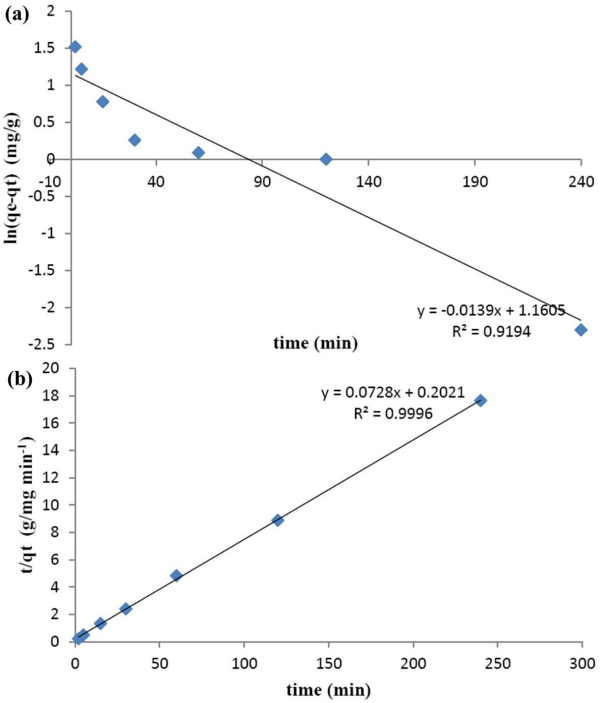

Fig. 4. Pseudo-first-order (a) and pseudo-second-order (b) kinetic plots for the adsorption of Cd^{2+} onto dry biomass of *Actinomucor* sp.

HNO_3 reduced adsorption capacity to amount of 24% and 52%, respectively. Pretreatment of adsorbent by autoclaves almost ineffective on the absorption capacity of cadmium (Fig. 6).

3.8. FTIR analysis

FTIR spectroscopy was done at the wavelength ranges of 400–4,000 cm^{-1} for the dry biomass of *Actinomucor* sp. before and after cadmium adsorption process. The location of resulted peaks was evaluated (Table 5 and Fig. 7). The comparison between the obtained peaks for the fungal biomass before and after the adsorption showed that the phosphate (P–O), carboxyl (COOH), hydroxyl (OH–), amine (N–H) and (–-N) groups are the main functional groups that connected to cadmium ions in the surface of *Actinomucor* sp. FTIR diagram for dry biomass before and after the cadmium ions adsorption process is presented in Fig. 7.

3.9. SEM analysis

SEM images of the *Actinomucor* sp. biomass were presented before (a) and after (b) Cd^{2+} biosorption (Fig. 8). These images clearly show the deformation and precipitation of bulky particles on the surface of *Actinomucor* sp. cells, which were absent on the surface of the biomass before cadmium treatment. This micrograph might prove that cadmium ions can be adsorbed on to the fungal cell surfaces.

3.10. Metal desorption and recovery of biosorbent

The result of biosorbent regeneration and cadmium release demonstrated that the biomass treatment with 0.1 M nitric acid at pH 2 for 2 h could desorb more than 87% of the cadmium from *Actinomucor* sp.

4. Discussion

Study of different fungal strains can lead to identification of the high potent strains for adsorbing heavy metals. Iskandar et al. [9] and Zafar et al. [20] reported that the screening of various ecosystems is an applicable method to identify new strains capable of adsorbing heavy metals. Here, for the first time, we screened *Actinomucor* sp. UTMC 5048 among 200 isolates from the contaminated soil which is a powerful fungal strain to adsorb cadmium ions with the adsorption capacity of 24.03 mg g^{-1}. Comparison between the q_{max} of this strain and the other biosorbents are presented in Table 6. Our results demonstrated that in comparison with the others fungal biosorbents, *Actinomucor* sp. is showing a high ability for Cd^{2+} sorption (Table 6). In addition, MIC results toward different metal ions showed that this strain is able to grow in the presence of various metal concentrations (Table 2). The importance of this issue is the presence of various metals in contaminated wastewater [21].

Adsorption isotherm models are used to assess the balance between the adsorption capacity and residual concentration of pollutants in the solid phase of the adsorbent and solution. However, the same isotherm models cannot be introduced for pollutant adsorption by the adsorbents since the model is dependent on the type of the contaminant and the adsorbent [12]. The results of Yan and Viraraghavan [22] showed that the adsorption of *Mucor rouxii* biomass for the metal ions including Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} follows the Langmuir isotherm model. This study results also indicated that the adsorption of cadmium ions by the adsorbent *Actinomucor* sp. follows the Langmuir isotherm model with the correlation coefficient (R^2 = 0.99). Calculation of R_L dissociation constant (R_L = 0.5–0.05) and b constant (b = 0.02) for the Langmuir isotherm and adsorption density constant of n (n = 0.29) for Freundlich isotherm showed that the process of cadmium ion adsorption by the introduced fungal biosorbent is eligible. Accordingly, the adsorption is unfavorable when the dissociation constant is $R_L > 1$ and is optimal if $0 > R_L > 1$ [23]. R_L constant was calculated (R_L = 0.5–0.05) for

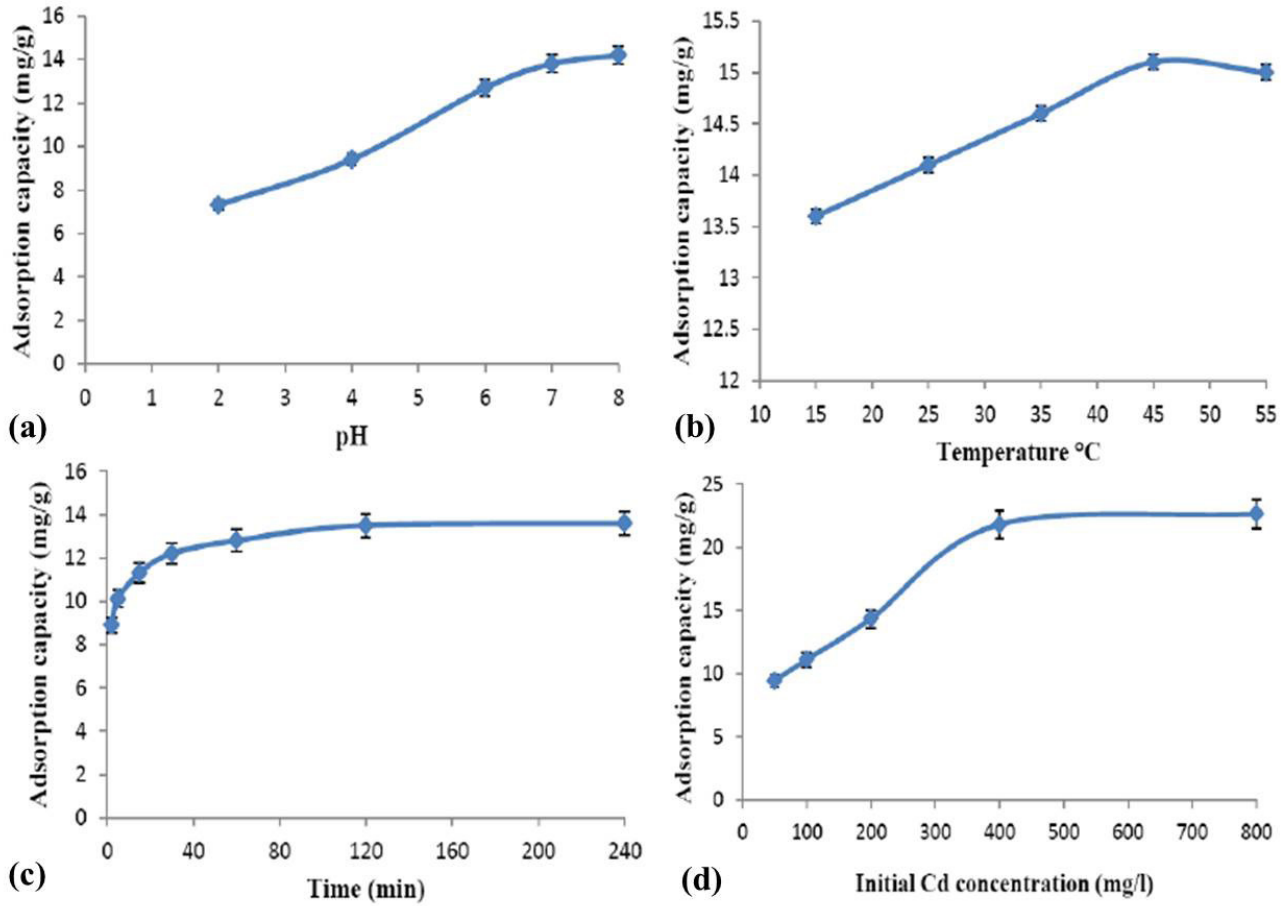

Fig. 5. Effect of pH (a), temperature (b), contact time (c) and initial concentration (d) on cadmium biosorption by the absorbent.

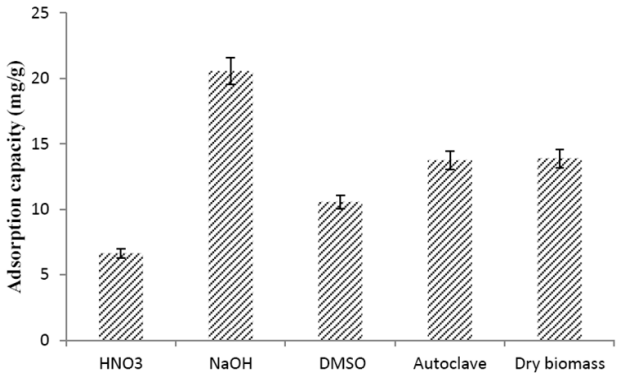

Fig. 6. Plot of pretreatment effect on the absorption capacity of Cd^{2+} by *Actinomucor* sp.

the adsorbent which indicates the legibility of the adsorption process.

Pseudo-first-order model cannot describe the overall adsorption process since the adsorption process is dynamic and follows the rules of an equilibrium reaction. In pseudo-second-order model both sites of adsorption and metal concentration are considered as factors affecting on adsorption rate [16], means that the rate of reaction in saturated condition is directly proportional to the number of active sites on the surface of the adsorbent [24]. Kinetic studies illustrated that cadmium adsorption by *Actinomucor* sp. fits pseudo-second-order model (Fig. 4).

The pH of the contaminated aqueous solution has a significant influence on the capacity for cadmium ions biosorption. The increase of pH would increase the negative charge on the surface of adsorbent, especially upon further increasing the pH the biomass surface, which decreases the electrostatic repulsion between negative charge of adsorbents and the positive charge of Cd^{2+} [25,26]. Yan and Viraraghavan [22] demonstrated that absorption capacity of cadmium by *M. rouxii* increased under the impact of rising pH. The temperature of the adsorption medium affects biosorption of metal ions. The increase of temperature by enhancement of collision rate and molecular diffusion or due to the availability of more active sites on the surface leads to increase absorption capacity of absorbent [27]. Sing et al. [28] reported that optimal absorption of cadmium by *Aspergillus niger* decomposed biomass was carried out at 30°C. The experimental results of Singh et al. [29] illustrated a contact time of 300 min were to be suitable for absorption of Pb(II) by agricultural residues. The amount of biosorption affected by the initial concentration of metal ions. This is because more Cd^{2+} is available at higher initial concentrations

which in turn may have provided higher driving force for the ions from the solution to the adsorbents [30]. The result of study Xu et al. [17] indicated biosorption of cadmium by an isolate related to *Penicillium chrysogenum*, as the Cd^{2+} concentration increased from 100 to 600 mg L^{-1}, the biosorption capacity increased from 36.21 to 77.99 mg g^{-1} dry biomass.

It has been shown that the alkali treatment of fungal biomass significantly increases the metal sorption. NaOH pretreatment can rupture cell walls and released polymers such as polysaccharides results in enhancement of adsorption sites [31,32]. Acid pretreatment significantly decreased bioadsorption of heavy metals, the remaining H^+ ions on the acidic pretreated biomass may change the biomass electronegativity, also DMSO detergent blocked the active sites of adsorption [32,33].

As presented in Fig. 8, the SEM analysis before and after Cd^{2+} adsorption in the strain showed that surface structure of absorbent has been changed due to cadmium uptake (Fig. 8).

FTIR spectroscopy method is used for the interaction examination between the functional groups engaged in a combination [34]. Almost all of the compounds with covalent bonds adsorb various frequencies of electromagnetic radiation in the infrared spectrum [34,35]. Binding the metal ions and other compounds to the functional groups of the adsorbent surface causes a shift and the restructuring of FTIR adsorption peaks of the functional groups in the chart. The study of Xu et al. [17] showed that the most important positions for cadmium uptake in fungus *P. chrysogenum* are hydroxyl, amines, carbonyl and sulfate groups [12]. A study was done on *A. niger* in 2015 by Sing et al. [28] for Pb^{2+} and Cd^{2+} and the same methods of FTIR spectroscopy and SEM were applied and showed high capacity of such microorganism to uptake heavy metals specially in synthetic solution. Another study also was conducted to investigate the ability of five various combinations of two adsorbents including *Eucalyptus cameldulensis* sawdust and *Arachis hypogea* shell powder, to remove Pb(II) from synthetic and lead acid batteries wastewater using batch and column mode [29]. The results of this study showed that the most active sites for cadmium uptake in the strain *Actinomucor* sp. are phosphate, carboxyl, hydroxyl and amine groups.

5. Conclusion

Our results showed that the strain *Actinomucor* sp. is a powerful adsorbent for cadmium ions from contaminated wastewater with a maximum adsorption capacity (q_{max}) of 24.03 mg g^{-1} and this fungal strain introduced for the first time as cadmium adsorbent and other heavy metals tolerant.

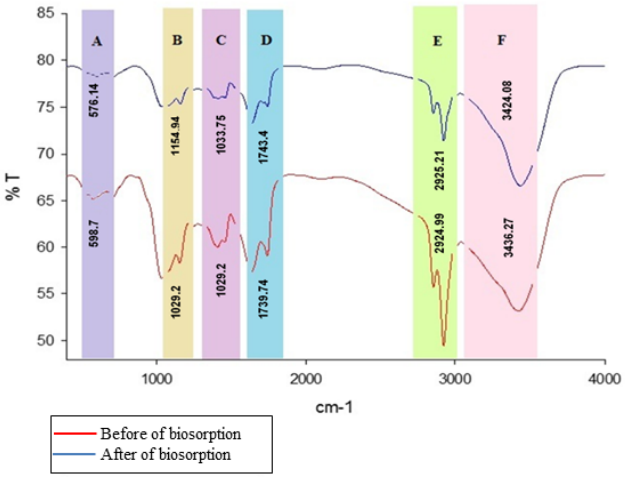

Fig. 7. FTIR spectra of *Actinomucor* sp. mycelia pellet before and after cadmium adsorption. A: P–O stretching, B: –CN (aliphatic amine), C: –CH bending/C–C stretching, D: N–H bending of primary amines, E: –CH stretching (alkanes) and F: –OH and/or –NH stretching.

Table 5
Peak locations in FTIR test and functional groups related to each peak

Functional group	Bond location (cm^{-1})		
	General bond location	Dry biomass of *Actinomucor* sp. before the Cd^{2+} adsorption	Dry biomass of *Actinomucor* sp. after the Cd^{2+} adsorption
(OH–) hydroxyl groups	3,500–3,200	3,424.08	3,436.27
N–H stretching	3,500–3,100	3,424.08	3,436.27
Primary and secondary amines			
C–H stretching	3,000–2,850	2,924.99	2,925.21
Carboxylic acid	3,400–2,800	2,856.9	2,855.76
Dimer OH			
C=O	1,740–1,730	1,739.74	1,743.4
N–H bending	1,640–1,560	1,632.94	1,634.56
Primary and secondary amines			
S=O sulfate	1,450–1,350	1,406.62	1,409.78
C–O stretching COOH	1,320–1,000	1,033.75	1,029.2
C–N stretching amines	1,230–1,030	1,154.94	1,029.2
P–O stretching	500–700	576.14	598.7

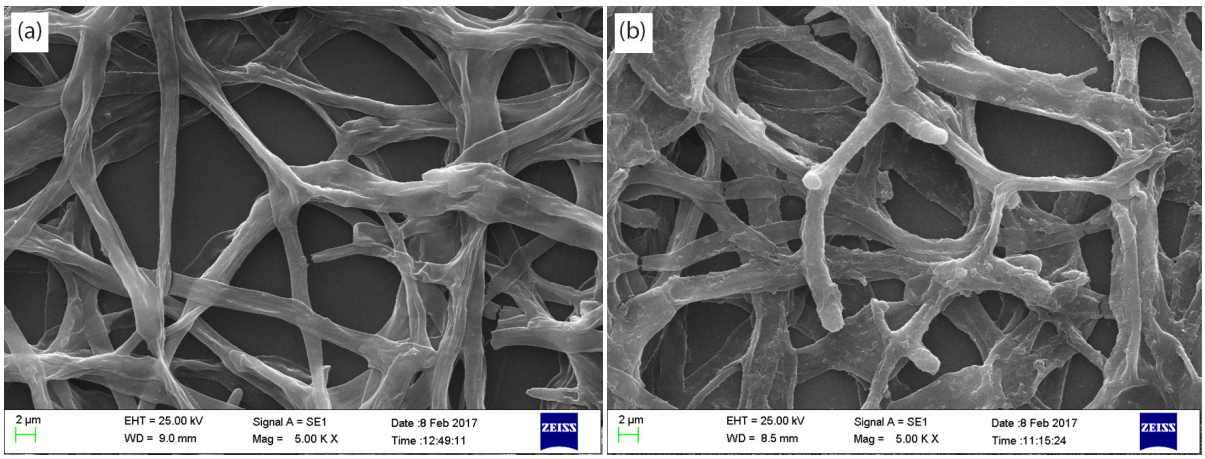

Fig. 8. SEM images of the *Actinomucor* sp. biomass (a) before and (b) after Cd^{2+} biosorption.

Table 6
Comparison between the maximum cadmium adsorption capacities (q_{max}) of *Actinomucor* sp. and the other biosorbents

Fungal strains	Adsorption capacity (q_{max}) (mg g^{-1})	Reference
Aspergillus niger	1.31	[37]
Mucor rouxii	20.31	[22]
Penicillium chrysogenum	21.5	[38]
Saccharomyces cerevisiae	35.5	[19]
Penicillium simplicissium	52.5	[39]
Actinomucor sp. UTMC 5048	24.09	This study

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Conflict of interest

The authors declare that they have no conflict of interest.

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