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Biosorption of cadmium ions using *Pleurotus ostreatus*: Growth kinetics, isotherm study and biosorption mechanism

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Abstract

The mycelial growth kinetics, cadmium biosorption capacity and main governing biosorption mechanism of *Pleurotus ostreatus* (oyster mushroom) have been determined in this study. The fungus mycelium exhibits a sigmoidal (S-shaped) growth curve in which the growth rates for the lag and exponential phases are 0.1 and 0.31 g/L.day, respectively. The grown fungus is subjected to elemental, infra-red and scanning electron microscopy-energy dispersive x-ray spectroscopy analyses while biosorption data are fitted to established adsorption isotherm models, namely, Langmuir, Freundlich and Dubinin-Radushkevich. It is strongly suggested that the main governing mechanism involved is chemisorption due to good fitting of biosorption data to Langmuir and Dubinin-Radushkevich models with possibility of involvement of both ion exchange and complexation. Data presented in the study are very useful for design of future pilot- or industrial-scale biosorption water purification system.

Running title: Biosorption of cadmium using *Pleurotus ostreatus*

Keywords: Pleurotus ostreatus; biosorbent; cadmium

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1. Introduction

Metal-laden industrial wastewaters are hazardous liquid wastes which have detrimental implications on the public health as well as surrounding ecological systems since metals are generally non-biodegradable and can bio-accumulate in living tissues. Over the past decades, the influx of these metal-laden wastewaters into the earth's surface environment has increased significantly due to large-scale global industrialization. These toxic metals include lead, cadmium, copper, nickel, chromium and mercury which originate from industries such as metal plating, paint and metal finishing.

One of the most utilized methods of metal ions removal from aqueous solution is precipitation (increase of solution pH via addition of basic substances, i.e. lime) but it has a significant drawback: large amount of undesired hydroxide sludge can be produced which necessitates further treatment and disposal. In view of this, alternative treatment processes such as metal adsorption using cheap adsorbents have been gaining popularity among water treatment researchers. Studies on usage of biological-based adsorbents (biosorbents) for removal of metal ions from aqueous solutions have intensified during the past decade due to their apparent advantages over conventional adsorbents. Biosorbents, in essence, are renewable, more cost-effective and exhibit high metal uptakes. Examples of these biosorbents include algae [1], *Rhizopus oryzae* [2] and orange peel [3]. Recently, fungi such as *Aspergillus niger* [4], *Penicillium janthinellum* [5] and *Agaricus macrosporus* [6] have been extensively studied by numerous researchers for biosorption of metals from aqueous solutions due to their relatively high removal efficiencies compared to biosorbents derived from plant or bacteria.

Although various fungal species have been investigated for possible utilization as metal biosorbents, studies on application of *Pleurotus ostreatus* as standalone biosorbent for metal removal from aqueous solution are comparatively scarce. The genus *Pleurotus* is a cosmopolitan group of mushrooms with highly nutritious values, therapeutic properties and various environmental and biotechnological applications [7]. *Pleurotus ostreatus* (generally known as oyster mushroom) is an edible fungus with good medicinal properties and widely cultivated in many countries around the world, namely, Germany, Korea, India and China. It shows strong laccase activity among edible mushrooms and is comparatively easy to culture in a medium. Recently, Pan and co-researchers [8,9] report on biosorption of cobalt and lead using *Pleurotus ostreatus* immobilized in calcium alginate and discover that such biosorbent system is capable of removing Pb (II) and Co (II) from aqueous solutions. As such, further studies on its usage within the field of wastewater treatment are justified. To the best of the authors' knowledge, an all-encompassing study on the preparation and growth kinetics of *Pleurotus ostreatus* and its subsequent application as standalone biosorbent for metal removal from aqueous solution is currently non-existent. Such study will ultimately be useful for wastewater treatment specialists seeking alternative renewable biosorbents that directly contribute to the global sustainable development initiatives.

The objectives of this study are to establish the mycelial growth kinetics of *Pleurotus ostreatus*, evaluate its cadmium biosorption capacity and determine its main governing biosorption mechanism. Cadmium is selected in this study because it is a toxic metal commonly present in wastewater effluent from a variety of industries, especially electroplating and paint industries. The grown fungus is subjected to several

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characterization analyses while the biosorption data are fitted to established adsorption isotherm models.

2. Materials and methods

2.1 Growth and preparation of *Pleurotus ostreatus* as biosorbent

Pleurotus ostreatus was obtained from C & C Mushroom Cultivation Farm located in the southern state of Johor, Malaysia. Mycelium of *Pleurotus ostreatus* was cultured in 1 L flasks containing 400 mL of malt extract medium in an incubator shaker (Qmax 5000, ThermoElectron, USA) at agitation speed 125 rpm and temperature 25°C. It was cultured for 21 days so that the stationary phase of the culture could be established. Mycelium growth was measured by means of dry cell weight (weight of cell devoid of moisture). For preparation of biosorbent, the mycelium was harvested and autoclaved for 15 minutes at 121°C and 18 psi and subsequently dried overnight in an oven at 60°C. It was then ground into powder and sieved to size ≤ 150 µm. The prepared biosorbent was stored in a desiccator.

2.2 Characterization

Elemental composition of the biosorbent was determined using Leco TruSpec 932 elemental analyzer while specific functional groups in the biosorbent were identified using Series 100 PerkinElmer Fourier Transform Infra-Red (FTIR) spectroscopy system. The

surface morphology of the biosorbent was examined using scanning electron microscopy-energy dispersive x-ray spectroscopy (SEM/EDX) system (LEO 1455 VP/EDX Oxford 300, UK).

2.3 Biosorption isotherm study

Stock solution of Cd^{2+} was prepared by dissolving analytical-grade anhydrous cadmium (II) chloride salt (Acros organics, USA) in deionized water (18.2 MΩ-cm). A total of 0.5 g biosorbent in 50 mL volume of Cd^{2+} solution (initial concentrations ranged from 10 to 80 mg/L) was agitated by means of an orbital shaker at 125 rpm and room temperature (25°C). Initial pH of the solution was fixed at 6 because it was determined in a preliminary study that the biosorbent had the highest adsorption capacity at this pH. In addition, this acidic pH value is deemed as appropriate due to two factors, namely, Malaysian wastewaters are prevalently acidic in nature and there is less interference with speciation changes of cadmium in the form of hydroxides (which is normally present in alkaline solutions). After a contact time of 10 minutes, the suspension was centrifuged at 1400 rpm (1024, Kubota, Japan) for 20 minutes and the supernatant was analyzed via atomic absorption spectrophotometer (AAS) (AAnalyst 800, Perkin Elmer, USA) with an air–acetylene flame and hollow cathode lamps. The equilibrium adsorption capacity was calculated using the following equation:

$$q_e = \frac{(C_0 - C_e)V}{M} \tag{1}$$

where q_e (mg/g) is the equilibrium adsorption capacity, C_0 and C_e are the initial and equilibrium concentrations (mg/L) of Cd^{2+} in solution, V (L) is the volume and M (g) is the weight of dry biosorbent.

3. Results and discussion

3.1 Growth of *Pleurotus ostreatus*

Growth of *Pleurotus ostreatus* mycelium at different growth phase has been investigated. The culture is initiated with 7 plugs of *Pleurotus ostreatus* culture from potato dextrose agar plate. Fig. 1 shows the growth curve of *Pleurotus ostreatus* mycelium with R^2 value of 0.9960 indicating good fit of the growth data to the sigmoidal (or S-shaped) model. In a previous study conducted by Wu et al. [10], similar curves were also observed for growth of *Pleurotus tuber-regium* mycelium with different carbon sources where lag phase only occurred for a few days. In our study, the biomass culture experiences lag growth for approximately 5 days before it enters into the exponential growth phase for the following 10 days. Day-15 of culture marks the onset of the stationary phase where it is harvested for preparation as biosorbent. The growth rates for the lag and exponential phases are 0.1 and 0.31 g/L.day, respectively, while the average dry biomass of per flask collected at day-15 is 1.58 ± 0.1070 g. These growth kinetics are significant data which can be utilized in the context of bulk production of the fungus for biosorption application.

3.2 Elemental analysis

Table 1 compares the elemental composition of *Pleurotus ostreatus* with other fungi. The amount of carbon of our fungus is quite similar to other types of fungi. The relatively high amount of nitrogen (5%) implies the probable presence of amine-based groups in *Pleurotus ostreatus.*

3.3 Biosorption isotherm study

Biosorption isotherm is an important component for the elucidation of how metal ions interact with the biosorbent (adsorption mechanism) and useful for optimizing the use of the biosorbent in batch process mode. Equilibrium is attained after just 10 minutes of contact time (i.e. cadmium concentration in solution remains constant after 10 minutes). This is confirmed in a preliminary experiment using repeated analysis of the cadmium concentration in the solution sampled once every 0.5 minutes for the entire duration of the batch adsorption process. The first two minutes of the preliminary experiment indicates cadmium removal of approximately 78% while subsequent 3, 5, 10, 15 and 30 minutes indicate cadmium removal of about 80, 84, 89, 89 and 89%.

Fig. 2 shows the plotted biosorption isotherm based on the batch adsorption process. The maximum amount of cadmium adsorbed on biosorbent is determined to be approximately 3.8 mg per gram of biosorbent. The obtained equilibrium data are also fitted to the Langmuir and Freundlich isotherm models. The Langmuir isotherm model [11] is

based on monolayer adsorption on active sites of the adsorbent and presented by the
following equation:

$$q_e = \frac{q_m K C_e}{1 + K C_e}$$ (2)

where q_m is the amount of cadmium ions adsorbed per unit mass of biosorbent
corresponding to complete monolayer coverage and K is the Langmuir constant. A linear
plot of (C_e/q_e) against C_e is created to provide values of q_m and K from the slope and the
intercept of the plot.

The Freundlich isotherm model [12] is expressed by the following equation:

$$q_e = K_F C_e^{1/n}$$ (3)

where K_F and n are constants. A linear plot of log q_e against log C_e is employed to provide
values of K_F and n from the intercept and slope of the plot.

The obtained Langmuir and Freundlich isotherm parameters are listed in Table 2. Our
biosorption data fit the Langmuir model considerably better than the Freundlich model
judging by the higher R^2 value for the former. This provides an initial indication that the
governing mechanism is chemisorption with high possibility of monolayer adsorption of
cadmium ions occurring on the surface of the biosorbent. It has been widely established
that the Langmuir adsorption isotherm assumes that adsorption occurs at specific sites
within the adsorbent [11]. Therefore, once the adsorbate occupies a site, no further
adsorption will take place at that site. It appears that other fungal biomass such as
Pycnoporus sanguineus [13] and *Mucor rouxii* [14] also rely on chemisorption for removal
of cadmium from aqueous solutions since their adsorption data also fit the Langmuir
isotherm better. With respect to the predicted q_m values, *Pleurotus ostreatus* exhibits

comparable cadmium biosorption capacity as compared to *Pycnoporus sanguineus* and *Mucor rouxii.*

3.4 Free energy of biosorption

Since it has been suggested via Langmuir model that there is high possibility of chemisorption being the governing biosorption mechanism, we feel it is appropriate to further substantiate the existence of this mechanism. As such, we fit our isotherm data to the Dubinin-Radushkevich (D-R) isotherm model [15] to determine the mean free energy of biosorption. The linearized form of D-R equation is expressed as the following [16]:

$$\ln q_e = \ln q_{m,DR} - \beta \varepsilon^2 \tag{4}$$

where $q_{m,DR}$ is maximum uptake amount of cadmium ions by biosorbent (mg/g), β is the constant linked to sorption energy (mol^2/kJ^2) and ε is the Polanyi potential which is expressed as $RT \ln(1+1/C_e)$, where R and T are the gas constant (kJ/mol·K) and temperature (K), respectively. The plot of $\ln q_e$ versus ε^2 results in a straight curve. The slope of the D–R plot provides the β constant value while q_e is calculated from the intercept of the plot. The mean free energy of biosorption, E can be approximated using the obtained β value from D-R isotherm via the following equation [17]:

$$E = 1/(2\beta)^{0.5} \tag{5}$$

E can be further elucidated as the energy needed to transfer one mole of cadmium ion (sorbate) to the surface from infinity (i.e. bulk fluidic environment). The value of E is utilized to determine the governing adsorption mechanism whereby an E value lesser than 8

kJ/mol indicates the predominance of physisorption mechanism while an E value higher than 8 kJ/mol indicates that chemisorption is the main governing mechanism [18]. The results of the equilibrium data fitting to the D-R model indicate a high R^2 value of 0.97 with $q_{m,DR}$ and E equal to 4.35 mg/g and 19.61 kJ/mol, respectively. The fact that our E value is higher than 8 kJ/mol further validates that chemisorption is the main governing biosorption mechanism in our study.

3.5 FTIR analysis

Fig. 3 shows the FTIR spectra of biosorbent before and after adsorption of cadmium ions while Table 3 lists the identified functional groups. The adsorption peak around 1638 cm^{-1} shifts to 1626 cm^{-1} after biosorption. This suggests that the free carboxyl groups have changed into carboxylate, a phenomenon which normally occurs during the reaction between cadmium and carboxyl [19]. Such interaction is consistent with the good fitting of biosorption data with Langmuir isotherm since presence of carboxyl directly implies site specific interactions (an archetypal notion assumed in the Langmuir equation). At pH 6, speciation of cadmium ions is predominant in the form of Cd^{2+} and there is the possibility of localized interactions with both free carboxyl and carboxylate groups. It is well-established that *Pleurotus ostreatus* contains cell walls formed by chitin molecules (long-chain polymer of *N*-acetylglucosamine) [20]. The identified functional groups; -OH, -NH and -CH$_3$ in our study provide further evidence to support this statement since these groups are some of the main constituents that form *N*-acetylglucosamine. Bhanoori and Venkateswerlu [21] report that the oxygen ring and the hydroxyl groups of *N*-

acetylglucosamine play a significant role in binding with Cd^{2+} ions via complexation. The aforestated shifts provide indication that the acidic groups, carboxyl and hydroxyl are predominant contributors in chitin-cadmium complex. Li et al. [30] suggest that on the basis of variations of the bands, peak values of FTIR spectra indicate the prevalence of chelating characteristics of metal biosorption onto carboxyl groups and this affords further evidence on the complexation mechanism proposed in our study.

3.6 SEM/EDX analyses

Figs. 4 and 5 show the micrographs and EDX spectra of biosorbent before and after biosorption. The surface appears to be rough and sponge-like which is indicative of a typical biomass texture. Subsequent to adsorption experiment, its surface experiences a slight change in morphology with 'muddy' deposits dominating the surface. Quite clearly, the chemisorption process and sudden change in external bulk fluidic environment have quite a significant effect on its morphology. Prominent C and O peaks from EDX spectra confirm our elemental analysis result. The EDX analysis also identifies the presence of potassium in the unused biosorbent which is not detected after biosorption. As expected, cadmium is detected after the biosorption process which suggests the possibility of existence of ion exchange occurrence between potassium and cadmium ions. As such, this result supports the findings from Bueno et al. [22] where they suggest the possibility of ion exchange mechanism in biosorption process in which metallic species such as Pb^{2+}, Cr^{3+} and Cu^{2+} ions have tendencies to replace K^+ on *Rhodococcus opacus*.

4. Conclusions

The growth kinetics and cadmium biosorption capacity of *Pleurotus ostreatus* have been established. The fungus exhibits a sigmoidal growth curve where the growth rates for lag and exponential phases are 0.1 and 0.31 g/L.day, respectively. Stationary phase is attained after day-15 of growth. The maximum biosorption capacity is approximately 3.8 mg per gram of biosorbent. The main governing mechanism is likely to be chemisorption with possibility of involvement of ion exchange and complexation. These data are vital since they can be used for scaling-up existing laboratory-scale biosorption system to a larger system which is more appropriate for water purification applications.

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Figure Captions

Fig. 1. Growth of *Pleurotus ostreatus*. Inset shows raw *Pleurotus ostreatus*.

Fig. 2. Biosorption isotherm for cadmium ions at 25°C.

Fig. 3. FTIR spectra of biosorbent before and after biosorption.

Fig. 4. Micrographs of biosorbent (a) before; and (b) after biosorption.

Fig. 5. EDX spectra of biosorbent (a) before; and (b) after biosorption.

Table 1

Comparison of elemental composition of *Pleurotus ostreatus* with other fungi.

Element	*Pleurotus ostreatus* (This study)	*Pleurotus ostreatus* [23]	*Penicillium* biomass [24]	*Cyathus bulleri* [25]
Carbon (%)	44.10	44.00	48.10	41.20
Hydrogen (%)	5.60	8.00	7.60	6.60
Nitrogen (%)	5.20	-	4.40	1.60
Oxygen (%) – approximated by difference	45.10	-	-	-
Sulfur (%)	0.20	-	-	-

Table 2

Langmuir and Freundlich isotherm parameters for biosorption of cadmium ions at 25°C and their comparison with other fungal biosorbents.

	Langmuir parameters			Freundlich parameters			
	q_m (mg/g)	K	R^2	K_F	n	$1/n$	R^2
Pleurotus ostreatus (This study)	4.05	0.33	0.98	1.00	2.33	0.43	0.79
Pycnoporus sanguineus[a] [13]	3.18	1.17	1	1.70	6.74	0.15	0.83
Mucor rouxii[b] [14]	8.46	5.93	0.87	6.34	5.56	0.18	0.69

[a] Biosorption at 30°C and pH 6.

[b] Biosorption at pH 5.

Table 3

Identification of functional groups in biosorbent via FTIR analysis.

Wavenumber (cm^{-1})		Identified groups	Reference(s)
Before biosorption	After biosorption		
3280	3268	-OH groups and -NH groups	Bayramoglu and Arica [26]
2924	2924	C-H stretching, -CH, $-CH_2$, $-CH_3$	Beuno et al. [22]; Akar et al. [27]
1638	1626	Carboxylate functional groups, carboxyl groups	Chen et al. [19]
1545	1541	N-H deformation	Bhanoori and Venkateswerlu [21]
1377	1376	Stretching of –COO–	Pavia et al. [28]
1151	1152	-C-O, carboxylic acid	Fereidouni et al. [29]
1020	1023	–C–O– or –C–N– groups	Akar et al. [27]; Beuno et al. [22]

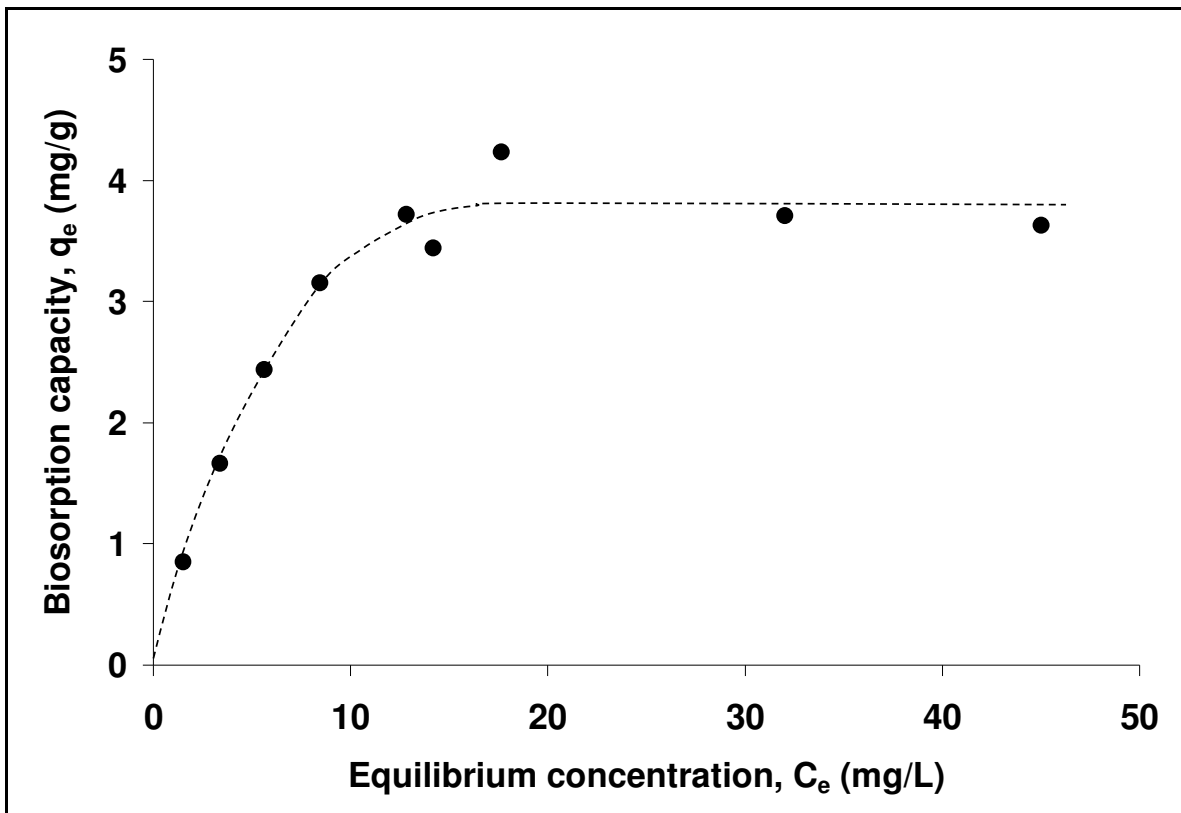**Fig. 1**. Growth of *Pleurotus ostreatus*. Inset shows raw *Pleurotus ostreatus*.

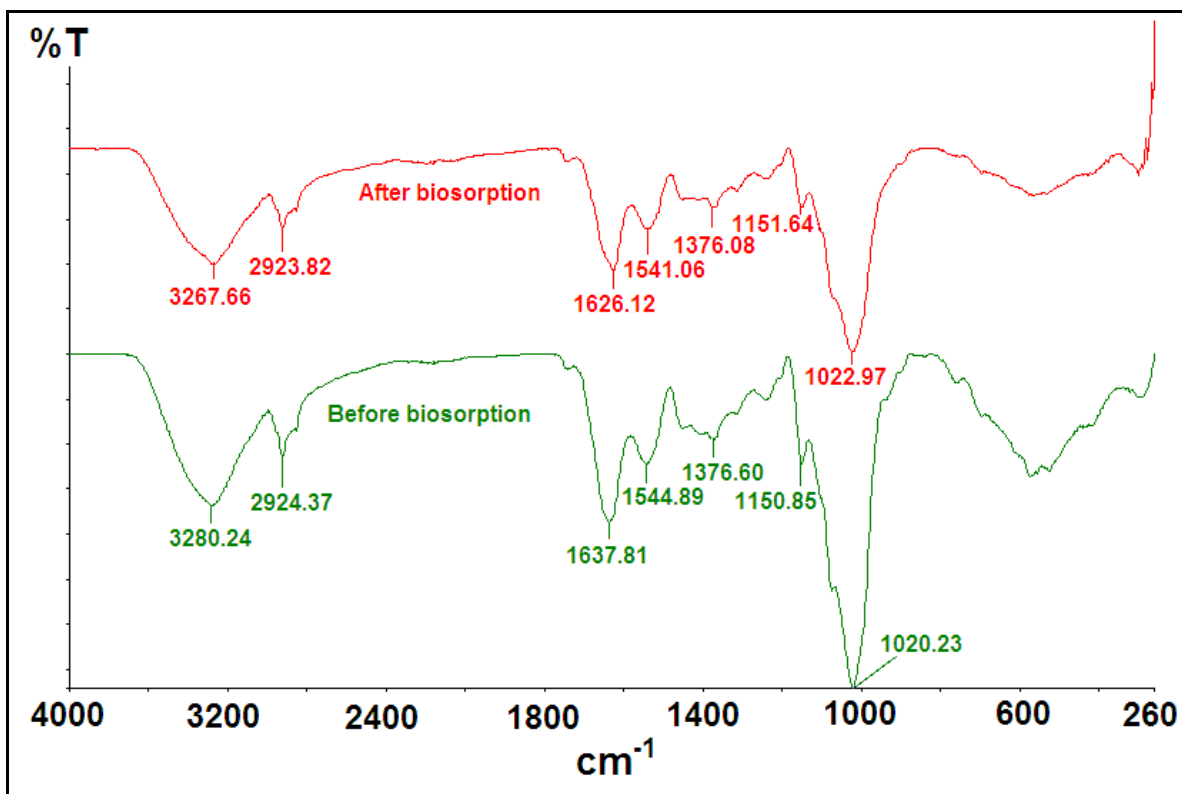**Fig. 2**. Biosorption isotherm for cadmium ions at 25°C.

Fig. 3. FTIR spectra of biosorbent before and after biosorption.
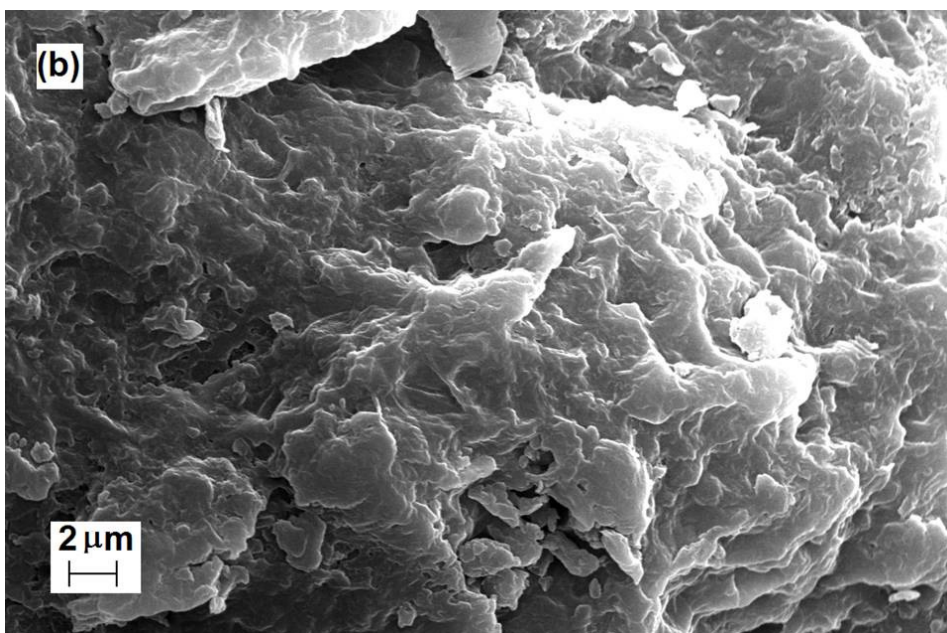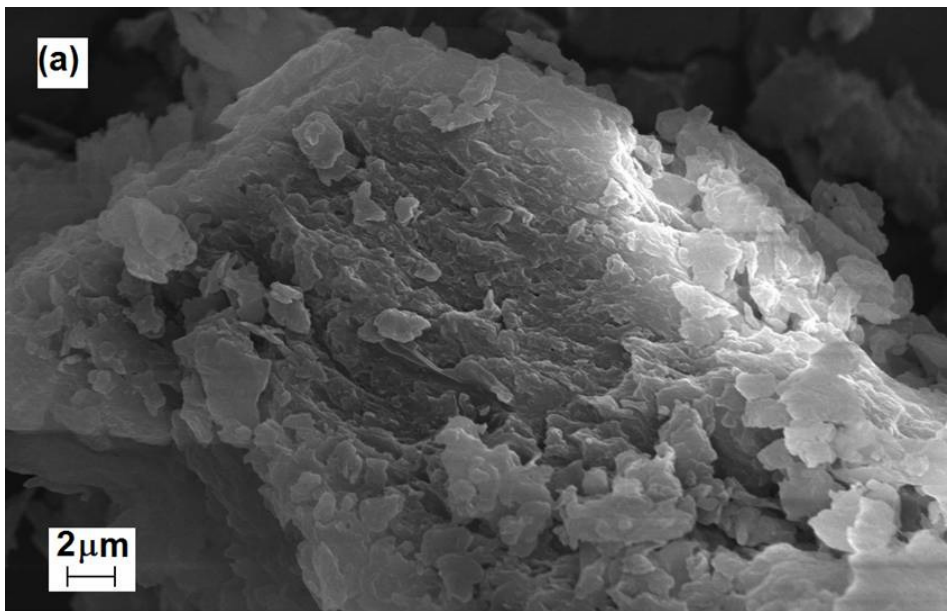
Fig. 4. Micrographs of biosorbent (a) before; and (b) after biosorption.
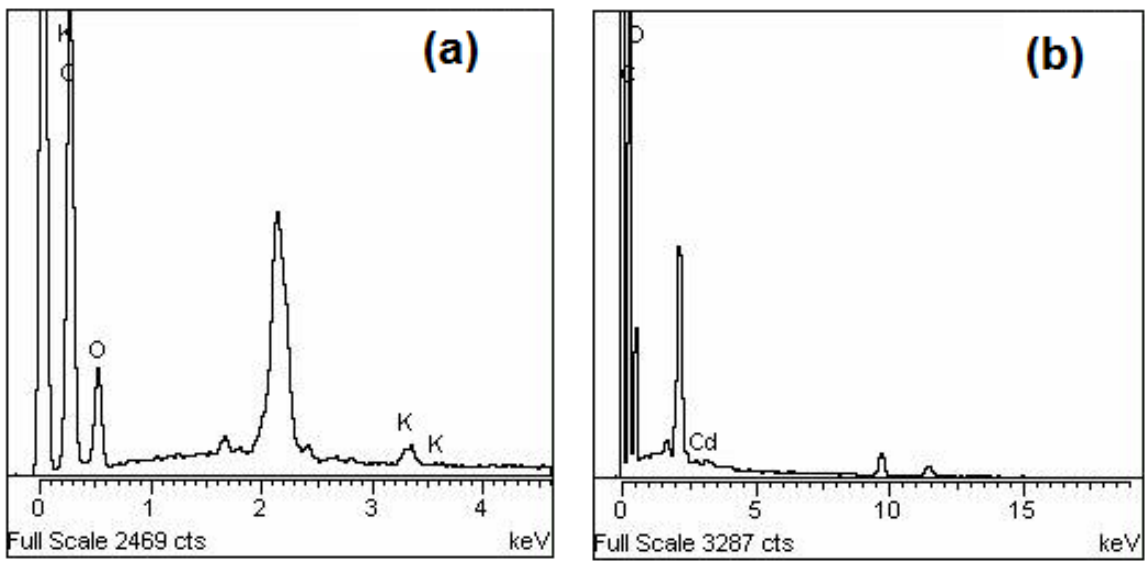
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