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Biosorption of Cd^{2+} by untreated dried powder of duckweed *Lemna aequinoctialis*

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ABSTRACT

The duckweed *Lemna aequinoctialis* was used as a biosorbent material for Cd^{2+} adsorption in this study. Influencing factors of Cd^{2+} adsorption by *L. aequinoctialis* in aqueous solution were investigated and the process of the Cd^{2+} biosorption was optimized. The results of single-factor experiments suggested that all the factors studied except temperature had significant effects on the removal efficiency of Cd^{2+} by *L. aequinoctialis*. Based on the results of single-factor experiments, optimization of the Cd^{2+} biosorption was performed by varying four independent parameters using the central composite design under response surface methodology. The optimal conditions for the maximum removal of Cd^{2+} were as follows: grain size of 150–200 mesh, stirring speed of 75 rpm, Cd^{2+} initial concentration of 40 mg/L, and sorbent concentration of 8 g dry matter/L. The maximum removal efficiency of 83.5% was obtained, which was in consistence with the predicted value of 83.6%. This process followed pseudo-second-order kinetics and the experimental data fitted well to Langmuir and Freundlich isotherm models. The maximum capacity of duckweed to adsorb Cd^{2+} was 33.0 mg/g, demonstrating that untreated dry powder of *L. aequinoctialis* represents a promising biosorbent for Cd^{2+} removal. Fourier transform infrared spectroscopy analysis indicated that the –OH groups of carbohydrate compounds and the $-NH_2$ groups of amide compounds may be the main groups involved in the adsorption of Cd^{2+} by *L. aequinoctialis*.

Keywords: Biosorption; Cadmium; Duckweed; *Lemna aequinoctialis*; Response surface methodology

1. Introduction

With the expansion of industry, increasing amounts of discharged wastewater are contaminating the environment with heavy metals. Cadmium is one of the most toxic metals and is harmful to living beings even in low dosages [1]. Kidney damage, renal disorder, and itai-itai disease are all caused by cadmium pollution [2]. The main sources of cadmium

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are mining, electroplating, and the waste of used nickel-cadmium batteries. Various methods that have been used to remove different heavy metals from wastewater have also been employed for the removal of cadmium, e.g. chemical precipitation [3], ion exchange [4], electrochemical filter [5], membrane technologies [6], and electro winning [7]. Although the methods are effective, all of them still have serious shortages. For example, chemical precipitation produces excessive sludge that requires further treatment; following ion exchange, organics and other solids were delivered into wastewater; in electro winning electrodes corrode and need to be replaced frequently [8,9]. High costs of equipment and high-operational costs limit the application of these methods.

Biosorption, as an alternative method, is becoming attractive to remove heavy metals from wastewater because biosorbents are cheap, easily available, and effective in heavy metals removal. Activated carbon is the most common sorbent used for the removal of heavy metals. However, the cost of this material is high [10]. Biosorbent materials were also made from different organisms, such as plant residues [11], yeast [12], fungus [13], and algae [14]. However, some of these materials have shortcomings. For example, micro-organisms need be pretreated using complex processes prior to be used as a biosorbent [12,15].

Also, higher plants have been used as biosorbents, e.g. Lemnaceae (duckweed) or *Pistia* [16]. Duckweed is a small, green floating family of aquatic plants which can be easily found around the world. It has a doubling time of 2–7 days [17]. The annual yields of the species *Spirodela polyrhiza* and *Lemna gibba* were reported to be 20.4 and 54.8 ton/ha in dry matter, respectively [18]. Therefore, duckweed can produce a large quantity of biomass. Furthermore, previous studies have showed that it is a promising biosorbent for the removal of heavy metals. Warenusantigul et al. reported the potential of one of the duckweed species (*S. polyrhiza*) as a biosorbent for the removal of the basic dye methylene blue from aqueous solution. The maximum adsorption capacity was 145 mg/g dry matter [19]. Miretzky et al. investigated biomass of three other macrophytes (*Spirodela intermedia*, *Lemna minor*, and *Pistia stratiotes*) for simultaneous removal of heavy metals. *L. minor* had the highest removal efficiency [16]. In general, only few researchers focus on the systematic use of duckweed as a biosorbent for removing cadmium.

The aim of the present study was to investigate and explore the potential application of *Lemna aequinoctialis* for the removal of the heavy metal cadmium (Cd^{2+}) from aqueous solution. The optimal conditions for maximum removal were studied using response surface methodology (RSM). The kinetic modeling, adsorption equilibrium, and the sorption mechanisms of Cd^{2+} were investigated. The results provided basic data for industrial application of duckweed as a biosorbent.

2. Materials and methods

2.1. Reagents

All chemicals used in the experiments were of AR grade. Ultrapure water (Milli-Q, Millipore, USA) was used for all solutions. Stock solutions of Cd^{2+} were prepared by dissolving $CdCl_2 \times 2.5H_2O$ (purity $\geqslant$ 99.0%) (Tianjin Jinbei Fine Chemical Co., Ltd., China). The solution pH was adjusted using 1 M HCl and 1 M NaOH solutions before the addition of biosorbent.

2.2. Biosorbent

L. aequinoctialis was collected in a wastewater treatment pond in rural area near Kunming, Yunnan province, China (E 102°42′ and S 25°02′) and collected in May 2012. The biomass was washed with distilled water, oven-dried at 60℃ until constant weight, and then pulverized in a grinder. The powder was stored in exsiccator at room temperature until use.

2.3. Single-factor experiments

Biosorption experiments were carried out in 250 mL flasks covered with parafilm and containing 100 mL of $CdCl_2$ solution. The flasks were stirred on incubator shakers (IS-RDS3, IncuShaker, USA). To investigate the effects of single factors on the removal efficiency, one factor was varied and all other were kept constant. The effects of the following factors were investigated in the ranges as given in brackets: contact time (10–300 min), pH (2–10), grain size (20–400 mesh), temperature (15–45℃), stirring speed (50–350 rpm), sorbent concentration (2–10 g/L), and initial Cd^{2+} concentration (2–300 mg/L). For an overview, cf. Table 1. After treatment, samples were withdrawn and centrifuged at 4,000 rpm for 5 min (ST 16R, Thermo, USA). The supernatant was analyzed by flame atomic adsorption spectrophotometry (Z-2300, Hitachi, Japan). A control experiment was carried out using the same solution in the absence of the plant material. Cadmium adsorption losses to the flask walls were negligible (data not shown). All adsorption experiments were performed in duplicate and all significance tests were carried out using SPSS 17.0 software.

Table 1
Experimental design of single-variable experiments

Experimental set	Contact time (min)	pH	Grain size (mesh[a])	Temperature (℃)	Stirring speed (rpm)	Sorbent concentration (g/L)	Cd^{2+} (mg/L)
1	**10–300**	7	100–300	25	200	5	20
2	120	**2–10**	100–300	25	200	5	20
3	120	5	**20–50** to **300–400**	25	200	2	20
4	120	5	100–300	**15–45**	200	2	20
5	120	5	100–300	25	**50–350**	6	20
6	120	5	100–300	25	200	**2–10**	20
7	180	5	100–300	25	200	5	**2–300**

[a] Unit conversion of mesh (20 mesh = 900 μm, 50 mesh = 355 μm, 100 mesh = 150 μm, 150 mesh = 100 μm, 200 mesh = 75 μm, 250 mesh = 63 μm, 300 mesh = 54 μm, and 400 mesh = 38.5 μm).

Note: The experimental sets were carried out by investigating one factor in a defined range (as indicated in bold letters) and keeping all other factors constant.

The removal efficiency was determined by the equation:

$$R = (C_0 - C_t)/C_0 \times 100\% \tag{1}$$

where R is the removal rate at each testing time, C_0 is the initial concentration of heavy metal (mg/L), and C_t is the concentration remaining in solution after each tested time of treatment (mg/L).

2.4. Kinetic modeling

The kinetic experiments were carried out as described for the experimental set No. 1 in Table 1.

The first-order rate expression of Lagergren based on solid capacity is generally expressed as follows:

$$\mathrm{d}Q_t/\mathrm{d}t = k_{ad}(Q_e - Q_t) \tag{2}$$

After integration and applying boundary conditions, $t = 0$ to $t = t$, and $Q_t = 0$ to $Q_t = Q_t$, the integrated form of Eq. (2) becomes as follows:

$$\ln(Q_e - Q_t) = \ln Q_e - k_{ad}t \tag{3}$$

where Q_e and Q_t are the amounts of adsorbed metal ions on the biosorbent (both in mg/g) at equilibrium and at time t, respectively. k_{ad} (min^{-1}) is the rate constant of first-order biosorption. A straight line of ln (Q_e−Q_t) vs. t suggests the applicability of this kinetic model. By fitting experimental data to Eq. (3), the equilibrium sorption capacity Q_e is obtained.

The pseudo-second-order equation is also based on the sorption capacity of the solid phase. The equation can be expressed as follows:

$$\mathrm{d}Q_t/\mathrm{d}t = k_2(Q_e - Q_t)^2 \tag{4}$$

After integration, Eq. (4) obtains the following form:

$$t/Q_t = 1/k_2Q_e^2 + t/Q_e \tag{5}$$

where k_2 ($\mathrm{g\,mg^{-1}\,min^{-1}}$) is the rate constant of second-order biosorption. A straight line of t/Q_t vs. t suggests the applicability of this kinetic model. By fitting Eq. (5) to the experimental data, Q_e and k_2 can be obtained.

The Q_t was determined by the equation:

$$Q_t = (C_0 - C_t)V/m \tag{6}$$

where V (L) is the volume of the solution and m (g) is the mass of the sorbent dose.

2.5. Equilibrium modeling

The equilibrium experiments were designed according to the experimental set No. 7 (Table 1).

The Langmuir equation can be expressed as:

$$Q_e = Q_m k_a C_e/(1 + k_a C_e) \tag{7}$$

where Q_m (mg/g) is the maximum adsorption capacity; k_a (L/mg) is a constant factor related to the affinity of the binding sites; and Q_e (mg/g) is the obtained results by testing not the calculated results through Eq. (6). The sorbent was separated and then digested with 8 mL HNO_3 and 2 mL H_2O_2 by microwave digestion system (Ethos Touch, MIESTONE, Italy) and Q_e was finally determined by AAS.

The empirical Freundlich equation based on adsorption to a heterogeneous surface is given by Eq. (8):

$$Q_e = k_F C_e^n \tag{8}$$

where k_F and n are the Freundlich constants representing adsorption capacity and adsorption intensity, respectively.

Correlation parameters can be obtained directly by non-linear fitting using Eqs. (7) and (8).

2.6. Optimization of cadmium biosorption

Optimum conditions for the biosorption of Cd^{2+} by dried duckweed material were determined by means of the central composite design (CCD) under RSM (Design-Expert 8.0.5.0 software).

The properties of the system could be described by the following quadratic equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \sum \beta_{ij} X_i X_j + \varepsilon \tag{9}$$

where Y is the predicted response; β_0 is the intercept term; β_i is the linear effect; β_{ii} is the squared effect; β_{ij} is the interaction effect; X_i and X_j are the dimensionless coded value of the variables; and finally, ε is a random error.

The range and level of variables investigated in this research are given in Table 2. A design of 30 experiments for the four variables formulated from sixteen factorial points, eight axial star points, and six replicate points at the central point was employed and analyzed using the quadratic model. The optimum values of the selected variables were obtained by solving the regression equation at desired values of the process responses as optimization criteria.

Table 2
Experimental ranges and levels of the independent variables

Factor	Symbol	Range and levels				
		−α (−2)	−1	0	1	+α (2)
Grain size (mesh)	X_1	75	125	175	225	275
Stirring speed (rpm)	X_2	0	75	150	225	300
Cd concentration (mg/L)	X_3	10	20	30	40	50
Sorbent concentration (g/L)	X_4	2	4	6	8	10

2.7. Analysis of sorbent by Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to probe the surface characteristics of *L. aequinoctialis* sorbent for elucidation of the sorption mechanisms of Cd^{2+}. Biosorbents (both before and after Cd^{2+} sorption) were dried to constant weight and then pressed into slices with KBr. Slices were observed by FTIR (Nicolet 6700, Thermo Fisher Scientific, USA).

3. Results and discussion

3.1. Single-factor experiments

For detailed experimental conditions, cf. the experimental sets 1–7 as described in Table 1.

3.1.1. Effect of contact time on cadmium biosorption

The removal efficiency strongly increased in the first 60 min of contact time and then increased much slower (Fig. 1(A)). This indicated that contact time had an important influence on the Cd^{2+} removal. The removal efficiencies were significantly different ($p < 0.01$) between 120 and 180 min but not between 180 and 300 min ($p > 0.05$). This suggested that in the second period of treatment, the amount of Cd^{2+} adsorbed onto the adsorbent was in a steady state of dynamic equilibrium with the amount of Cd^{2+} desorbed from the adsorbent. Therefore, 180 min can be defined as the time of treatment required to reach equilibrium.

3.1.2. Effect of pH on cadmium biosorption

As shown in Fig. 1(B), the removal efficiency strongly increased ($p < 0.01$) from 6.60 to 75.59% between pH 2 and 3, and remained constant ($p > 0.05$) at approximately 75% at the pH range of 3–9; then, there was a significant decrease ($p < 0.01$) at pH 10. The removal efficiencies at pH 2 and 10 were therefore significantly lower compared with the efficiencies in the range between pH 3 and 9, which agreed with the results reported in the adsorption of other heavy metals by fern biomaterial [20]. It might be due to the influence of excess H^+, H_3O^+, and OH^- on sorbent surface. At very low pH (pH 2), there was an excess of H^+ (or H_3O^-) ions which may compete for adsorption sites on sorbent surface. At high pH, the Cd^{2+}, closely associated with high amount of OH^-, might not be adsorbed efficiently by the functional groups with negative charge [21,22]. Consequently, the pH range of 3–9 was determined the optimal pH condition of Cd^{2+} adsorption by *L. aequinoctialis*.

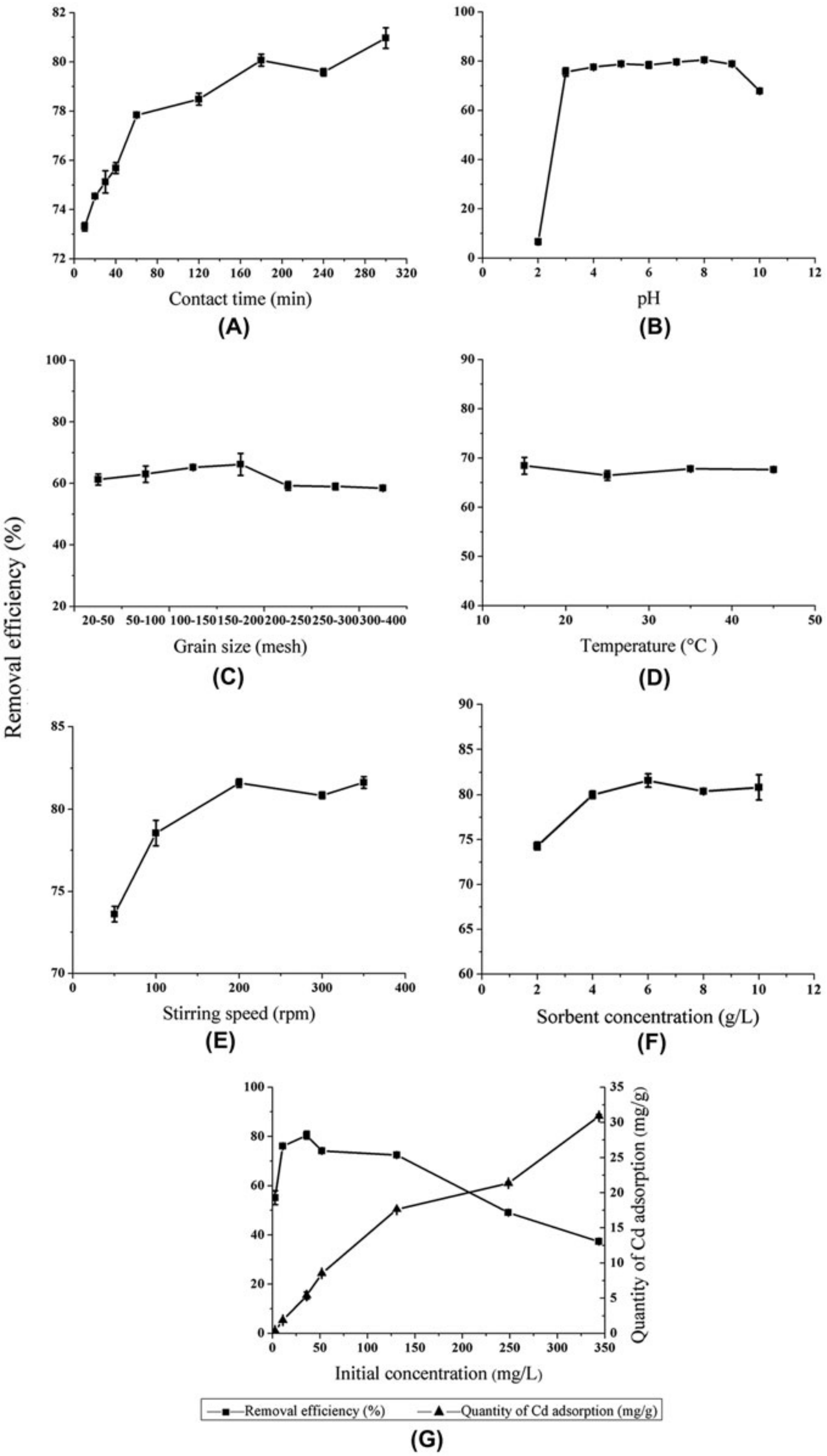

Fig. 1. Effects of different variables on the removal efficiency of Cd^{2+} by *L. aequinoctialis*. The effects of (A) contact time, (B) pH, (C) grain size, (D) temperature, (E) stirring speed, (F) sorbent concentration, and (G) initial concentration on the removal efficiency.

3.1.3. Effect of grain size of sorbent on cadmium biosorption

Although the removal efficiency at 150–200 mesh was significantly higher than that at 300–400 mesh ($p = 0.05$), the removal efficiency of Cd^{2+} showed no significant difference ($p > 0.05$) at other grain sizes of adsorbent (Fig. 1(C)). Al-Masri et al. used poplar leaves and branches as biosorbents to remove cadmium, lead, and uranium from aqueous solution. The results showed a reduction in the biosorption of Cd^{2+}, Pb^{2+}, and U^{6+} with the decreasing grain size of leaf biomass while there was no effect on uptake of Cd^{2+} by branches [23]. This may be due to different surfaces of sorbents.

3.1.4. Effect of temperature on cadmium biosorption

The Cd^{2+} removal efficiency for the range of 15–45℃ did not show any statistical difference ($p > 0.05$) (Fig. 1(D)). However, previous reports have described the effect of temperature on heavy metal adsorption to be controversial. The study from Rao et al. showed the adsorption of cadmium by leaf powder of *Psidium guvajava* L. was endothermic, since the higher temperature could increase the removal efficiency of cadmium [24]. Aksu studied that cadmium adsorption by *Chlorella vulgaris* was normally exothermic thus the extent of adsorption generally increases with decreasing temperature [25]. Martins et al. reported that in the range of 298–328 K, the increase of temperature did not significantly affect biosorption of lead on *Sargassum* sp. [26]. The inconsistency regarding the influence of temperature on biosorption may indicate that the temperature could alter the adsorption mechanism of metal ions in the adsorption process by some types of sorbents.

3.1.5. Effect of stirring speed on cadmium biosorption

Flasks were shaken in order to intensify the contact of Cd^{2+} with the sorbent and to shorten the time required to reach an equilibrium state. As shown in Fig. 1(E), the removal efficiency increased with the increasing stirring speed no more than 200 rpm. Then, an equilibrium was reached at ⩾200 rpm, which indicated that 200 rpm was sufficient to avoid effects of external diffusion on the Cd^{2+} absorption and promote the adsorption of Cd^{2+} by *L. aequinoctialis*.

3.1.6. Effect of sorbent concentration on cadmium biosorption

As shown in Fig. 1(F), with the increase of sorbent concentrations, the removal efficiency significantly increased ($p < 0.01$) from 74.28% at 2 g/L to 79.99% at 4 g/L. And, subsequently, it became relative stable; there was no significant difference ($p > 0.05$) when the sorbent concentration was over 4 g/L.

3.1.7. Effect of initial concentration of cadmium on cadmium biosorption

The effect of initial concentration of Cd^{2+} on the removal efficiency was very evident (Fig. 1(G)). With the Cd^{2+} concentration being increased, the removal efficiency increased at the beginning and reached the maximum of 80.5% at 35.9 mg/L, thereafter decreased gradually. High initial concentration would give rise to the supersaturation of sorbent and then a decrease of removal efficiency. The Cd^{2+} adsorption by *L. aequinoctialis* exhibited almost linear increase with the increasing initial concentration,

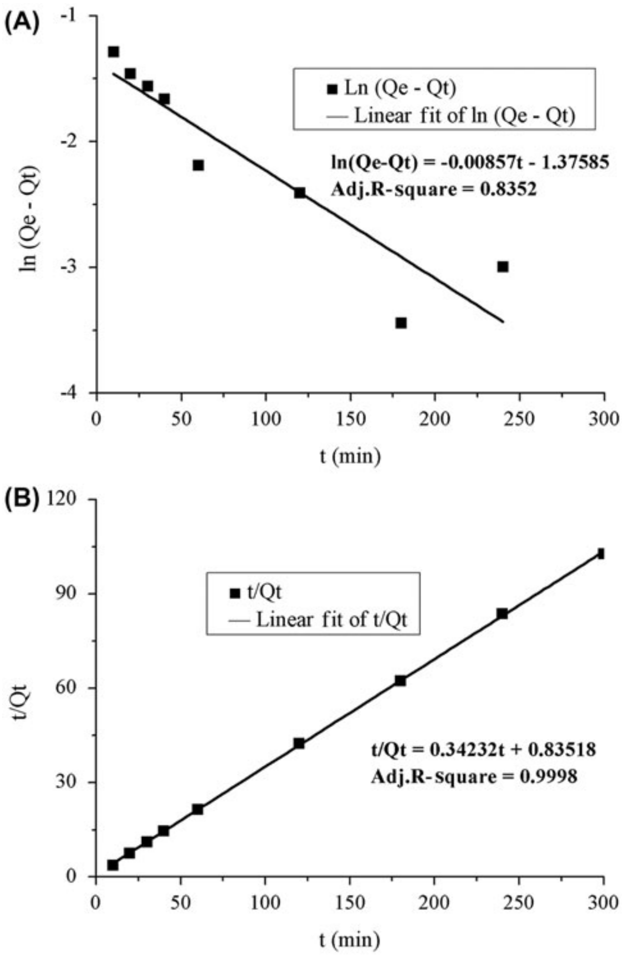

Fig. 2. The Larergren pseudo-first-order (A) and pseudo second-order (B) biosorption kinetics of Cd^{2+} on dried plant material from *L. aequinoctialis*.

that was due to the initial concentration provided an important driving force to overcome all mass transfer resistances of Cd^{2+} between the aqueous and solid phases [27].

3.2. Adsorption kinetics

To characterize the mechanism of biosorption and potential rate controlling steps such as mass transport or chemical reaction processes, two different kinetic models were tested by fitting the relevant equations to the experimental data. Linear and non-linear regressions were used to determine the best-fitting kinetic models [28]. Comparing the two procedures, we found that linear fitting described the experimental data better (data not shown). The low correlation coefficient (R^2) of 0.8352 obtained from the linear fitting of Eq. (3) indicated the adsorption process did not follow the Larergren first-order kinetics (Fig. 2(A)). However, a straight line obtained by plotting t/Q_t vs. t (Fig. 2(B)) demonstrated that the pseudo-second-order equation fitted the experimental results well, resulting in $R^2 = 0.9998$. The theoretical Q_e of 2.921 mg/g for *L. aequinoctialis* was almost identical with the experimental data (2.918 mg/g). The constant k_2 of $0.1452\,\mathrm{g\,min^{-1}\,mg^{-1}}$ was obtained from the slope and intercept of the line. Higher values of k_2 mean faster removal rates. These results provided the basic data for the design of an adsorption systems using dried material of *L. aequinoctialis*.

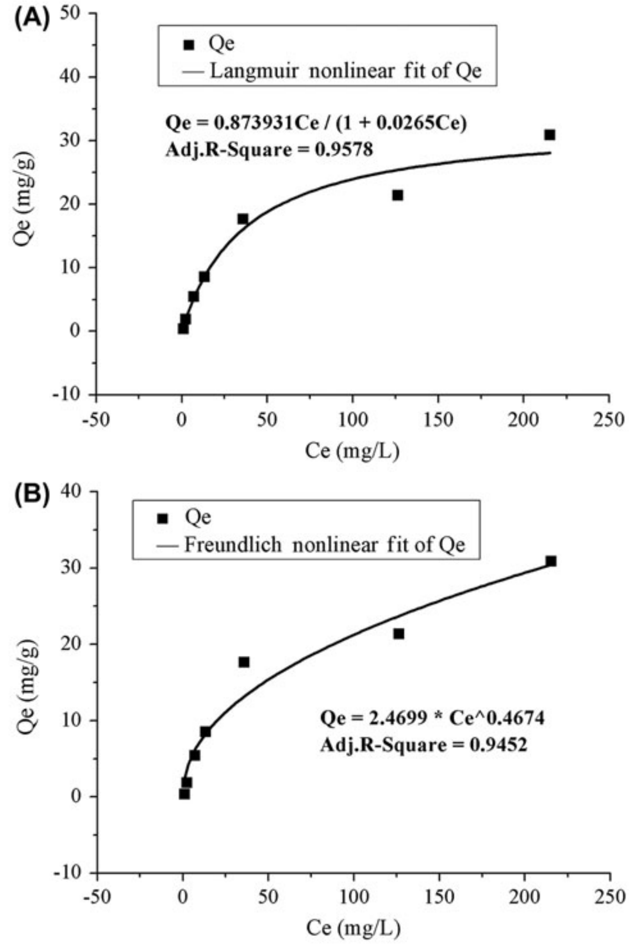

Fig. 3. Biosorption isotherms of Cd^{2+} based on the Langmuir (A) and Freundlich (B) models.

3.3. Adsorption isotherms

Linear and non-linear regressions were also used to determine the best-fitting adsorption isotherms, and the results suggested that non-linear fitting described the experimental data better than linear fitting (data not shown). The adsorption isotherms fitted well to the Langmuir and Freundlich isotherms with calculated R^2 values of 0.9577 and 0.9446, respectively (Fig. 3). Correlation parameters based on non-linear fitting equation were obtained. The maximum cadmium adsorption capacity (Q_m) and affinity constant (k_a) were 32.98 mg/g and 0.0265 L/mg, respectively. This magnitude of Q_m and k_a reflected high maximum Cd^{2+} adsorption capacity and strong bonding of Cd^{2+} to the *L. aequinoctialis* material, respectively. The values of the constants of k_F and n for Freundlich isotherm were 2.47 and 0.467, respectively. This implied easy adsorption of Cd^{2+} from wastewater with both high adsorption capacity and high adsorption affinity of dried *L. aequinoctialis* material. The Q_m value has often been used to compare the adsorption capacity of different sorbents (Table 3). This comparison demonstrates that *L. aequinoctialis* is a potential biosorbent for Cd^{2+} because of its high maximum cadmium adsorption capacity.

3.4. Optimization by RSM

According to the results of the single-factor experiments, grain size (X_1), stirring speed (X_2), initial concentration (X_3), and sorbent concentration (X_4) were selected as the four variables and the removal efficiency was the response for the CCD. The results are presented in Table 4. Table 5 shows the analysis of variance (ANOVA) for the response surface quadratic model. The F-value of 10.89 and the value of "Prob > F" less than 0.0001 indicated that the model terms were statistically significant. In accordance with

Table 3
Comparison of the maximum cadmium adsorption capacity (mg/g dry weight) of low cost sorbents

Adsorbents	pH	Temperature (℃)	Grain size (mm)	Adsorption capacity Q_m (mg/g)	Adsorption affinity k_a (L/mg)	References
Cystoseira baccata	4.5	25	0.5–1	77.56	0.0978	[29]
Scenedesmus obliquus	6.0	30	–	68.60	0.1011	[30]
Lemna aequinoctialis	5.0	25	0.15	32.98	0.0265	This study
Gelidium	5.3	20	0.25–1	18.00	0.19	[27]
Rice straw	5.0	25	<0.5	13.89	0.066	[31]
Lentinus edodes	5.0–6.0	25	–	6.45	0.0592	[32]
Pistia stratiotes	6.0	–	2	4.16	0.5266	[16]
Lemna minor	6.0	–	2	3.71	3.948	[16]

Table 4
Experimental design and results of the responses

Run order	Real (coded) values				Removal efficiency (%)	
	X_1	X_2	X_3	X_4	Experimental	Predicted value
1	125 (−1)	75 (−1)	40 (1)	4 (−1)	77.95	77.13
2	175 (0)	150 (0)	50 (2)	6 (0)	81.48	80.49
3	125 (−1)	225 (1)	20 (−1)	4 (−1)	81.88	82.39
4	125 (−1)	225 (1)	20 (−1)	8 (1)	83.76	81.58
5	175 (0)	150 (0)	30 (0)	6 (0)	82.00	80.06
6	175 (0)	150 (0)	30 (0)	2 (−2)	74.17	75.63
7	125 (−1)	75 (−1)	20 (−1)	4 (−1)	79.06	78.07
8	175 (0)	300 (2)	30 (0)	6 (0)	78.83	79.28
9	75 (−2)	150 (0)	30 (0)	6 (0)	74.83	74.99
10	225 (1)	225 (1)	20 (−1)	8 (1)	72.00	72.69
11	175 (0)	150 (0)	30 (0)	6 (0)	80.00	80.06
12	175 (0)	150 (0)	30 (0)	6 (0)	81.00	80.06
13	125 (−1)	75 (−1)	40 (1)	8 (1)	80.90	81.47
14	275 (2)	150 (0)	30 (0)	6 (0)	63.33	63.76
15	175 (0)	0 (−2)	30 (0)	6 (0)	80.33	80.47
16	225 (1)	225 (1)	40 (1)	8 (1)	74.23	74.75
17	225 (1)	75 (−1)	40 (1)	4 (−1)	73.08	74.80
18	225 (1)	75 (−1)	20 (−1)	8 (1)	74.12	73.98
19	175 (0)	150 (0)	30 (0)	10 (2)	81.17	80.29
20	225 (1)	225 (1)	20 (−1)	4 (−1)	73.41	72.37
21	225 (1)	75 (−1)	20 (−1)	4 (−1)	74.12	72.59
22	175 (0)	150 (0)	10 (−2)	6 (0)	77.78	79.36
23	225 (1)	75 (−1)	40 (1)	8 (1)	80.90	80.27
24	225 (1)	225 (1)	40 (1)	4 (−1)	71.41	70.36
25	125 (−1)	225 (1)	40 (1)	4 (−1)	77.56	77.24
26	175 (0)	150 (0)	30 (0)	6 (0)	77.00	80.06
27	125 (−1)	225 (1)	40 (1)	8 (1)	79.10	80.50
28	175 (0)	150 (0)	30 (0)	6 (0)	78.00	80.06
29	175 (0)	150 (0)	30 (0)	6 (0)	82.33	80.06
30	125 (−1)	75 (−1)	20 (−1)	8 (1)	77.41	78.34

Table 5
The results of ANOVA for the response surface of the quadratic model

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value Prob > *F*
Model	495.64	14.00	35.4	10.89	<0.0001
Residual	48.79	15.00	3.25	–	–
Lack of fit	25.38	10.00	2.54	0.54	0.8084
Pure error	23.41	5.00	4.68	–	–

it, the “Lack of fit *F*-value” of 0.54 and *p*-value of 0.8084 indicated that the lack of fit was not significant, which showed that the quadratic model was valid for the present study. R^2 was 0.9104, which showed that this regression was statistically significant and only 9% of the total variations was not explained by this model. The value of adjusted R^2 was 0.8268, indicating the high degree of correlation between the observed and the predicted values. Table 6 presents the results of the second-order response surface model in the form of ANOVA. In this case, the terms of X_1, X_4, X_1X_2, X_2X_3, X_3X_4, and ${X_1}^2$ had significant effects on the removal of Cd^{2+} as their *p*-values were less than 0.05 (Table 6).

By applying the multiple regression analysis to the experimental data, a second-order polynomial model (Eq. (10)) in a coded level was obtained, which could explain the role of each variable and its interaction in the process of Cd^{2+} removal:

$$\begin{aligned} Y = 80.06 - 2.81X_1 - 0.30X_2 + 0.28X_3 + 1.16X_4 \\ - 1.13X_1X_2 + 0.79X_1X_3 + 0.28X_1X_4 \\ - 1.05X_2X_3 - 0.27X_2X_4 + 1.02X_3X_4 - 2.67X_1^2 \\ - 0.044X_2^2 - 0.033X_3^2 - 0.52X_4^2 \end{aligned} \quad (10)$$

The optimized data as given by the quadratic model under optimized condition are as follows: $X_1 = 170.17$ mesh, $X_2 = 75$ rpm, $X_3 = 40$ mg/L, and $X_4 = 8$ g/L and the maximum removal efficiency of Cd^{2+} was predicated to be 83.6%.

Confirmatory experiments were performed in quadruplicate tests. Under the predicted optimized condition, the experimental average of removal efficiency was obtained as 83.5 ± 0%, which was in accordance with the predicated value and further verified the model as effective and reliable.

The entire relationship between two variables and response can be described by three-dimensional response surfaces (Fig. 4) generated from the quadratic model. Statistical analysis indicated that the removal efficiency was significantly affected by grain size and sorbent concentration. Moreover, significant interaction effects were observed for grain size and stirring speed, stirring speed and initial concentration, and initial concentration and sorbent concentration (Fig. 4 and Table 6).

3.5. *FTIR analysis of* L. aequinoctialis *before and after* Cd^{2+} *adsorption*

The FTIR analysis allowed identification of the chemical groups involved in the sorption of heavy

Table 6
Regression analysis using 2^4 factorial CCDs for *L. aequinoctialis*

Factor	Coefficient estimate	Standard error	*F*-value	*p*-value
Intercept	80.06	0.74	–	–
X_1	−2.81	0.37	58.13	<0.0001
X_2	−0.3	0.37	0.66	0.4303
X_3	0.28	0.37	0.59	0.4556
X_4	1.16	0.37	10.01	0.0064
X_1X_2	−1.13	0.45	6.34	0.0237
X_1X_3	0.79	0.45	3.04	0.1018
X_1X_4	0.28	0.45	0.39	0.5416
X_2X_3	−1.05	0.45	5.47	0.0336
X_2X_4	−0.27	0.45	0.35	0.5611
X_3X_4	1.02	0.45	5.11	0.0391
${X_1}^2$	−2.67	0.34	60.1	<0.0001
${X_2}^2$	−0.044	0.34	0.017	0.899
${X_3}^2$	−0.033	0.34	0.009	0.924
${X_4}^2$	−0.52	0.34	2.31	0.1491

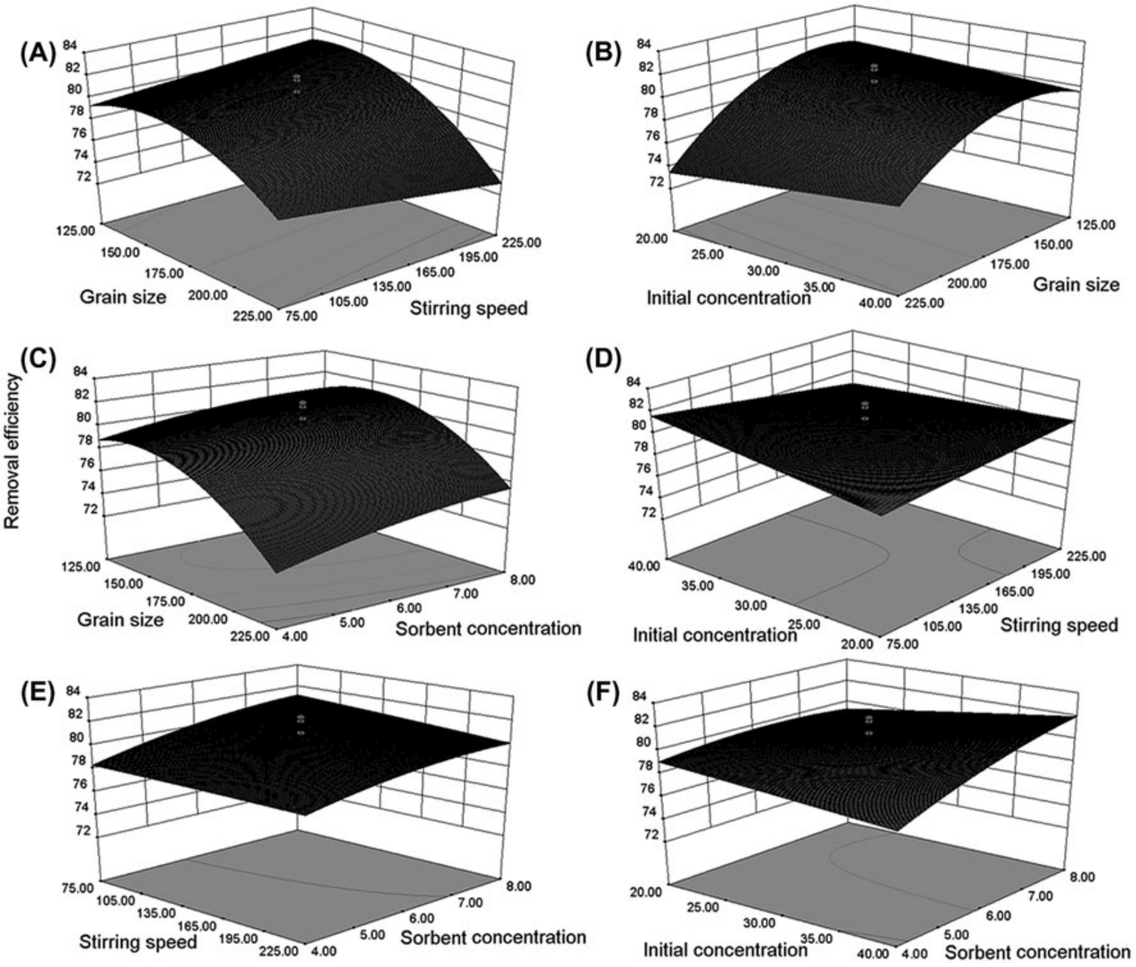

Fig. 4. 3-D plot of Cd^{2+} adsorption by *L. aequinoctialis*. The removal efficiency was shown in dependence of the following parameters: (A) grain size and stirring speed, (B) grain size and initial concentration, (C) grain size and sorbent concentration, (D) stirring speed and initial concentration, (E) stirring speed and sorbent concentration, and (F) initial concentration and sorbent concentration.

metals (Fig. 5). The broad and strong band ranging from 3,200 to 3,500 cm^{-1} might be due to the overlapping of –OH- and –NH-stretching bands (Peak 1). The peak at 2,926 cm^{-1} was attributed to stretching vibration of C–H ($\upsilon_{\text{C–H}}$) from saturated hydrocarbonds (Peak 2). The absorbance at wave numbers of 1648.4, 1541.8, and 1404.8 cm^{-1} could be assigned to the presence of a –CO/NHR group corresponding to stretching vibrations of carbonyl double bonds ($\upsilon_{\text{C=O}}$), rocking vibrations of nitrogen–hydrogen single bonds ($\delta_{\text{N–H}}$) and stretching vibrations of carbon–nitrogen single bonds ($\upsilon_{\text{C–N}}$), respectively (Peaks 3, 4, and 5). The peak at the wave number of 1054.9 cm^{-1} was contributed by the stretching vibrations of carbon-oxygen single bonds ($\upsilon_{\text{C–O}}$) (Peak 6). The characteristic peaks in the range of 500–800 cm^{-1} indicated that starch or some other sugars might be part of *L. aequinoctialis* constituents (Peak 7). Comparing the *L. aequinoctialis*

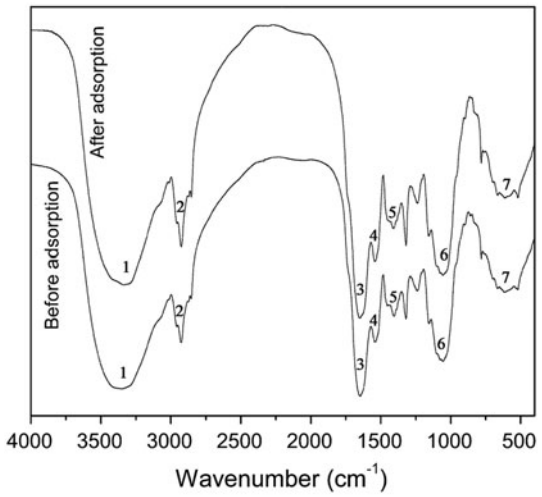

Fig. 5. FTIR spectra of the *L. aequinoctialis* samples before and after the biosorption of Cd^{2+}.

before and after Cd^{2+} adsorption, some shifts were observed in the location and absorbance of typical peaks. The –OH or –NH stretch band was shifted from 3349.6 to 3334.9 cm^{-1}, implying that the biosorption involved –OH or –NH deformation. The stretching vibration of the C–N bonds at 1404.8 cm^{-1} shifted to 1410.0 cm^{-1} and the vibration intensity reduced after the Cd^{2+} adsorption onto the *L. aequinoctialis*, which indicated that amide compounds were involved in the Cd^{2+} adsorption. The subtle changes of peak shape at the wave number of 1054.9 cm^{-1} further confirmed that hydroxyl group participated in chelation reactions. In the range of 500–800 cm^{-1}, almost all the vibration frequency of infrared bands appeared to decrease or increase apart from the peak at wave number of 698.1 cm^{-1}, demonstrating that the functional groups of the starch or some other sugars might have undergone chemical reactions.

4. Conclusion

Like most other biosorbents, the process of Cd^{2+} biosorption by *L. aequinoctialis* follows the pseudo-second-order kinetics equation and could be fitted both in terms of the Langmuir and Freundlich models. The maximum cadmium adsorption capacity of 33.0 mg/g by untreated dried *L. aequinoctialis* material was obtained from the Langmuir isothermal equation. This demonstrates that dried *L. aequinoctialis* is a promising biosorbent. The FTIR analysis indicated that the –OH groups of carbohydrate compounds and the $–NH_2$ groups of amide compounds may be the main groups involved in the adsorption of Cd^{2+} by *L. aequinoctialis*. According to the results of single-factor experiments, we optimized the Cd^{2+} adsorption by *L. aequinoctialis* using CCD. In order to reach the maximum cadmium removal, the following parameters were obtained in this study: grain size of 150–200 mesh, stirring speed of 75 rpm, Cd^{2+} initial concentration of 40 mg/L, and sorbent concentration of 8 g/L. Under the optimized conditions, the Cd^{2+} removal efficiency of 83.5% was in ideal agreement with that predicted by the model (83.6%). *L. aequinoctialis* is readily available in China as in many other countries [33], so it can easily be used for treating wastewater polluted by cadmium. The results presented here are useful for the practical application of *L. aequinoctialis* as a biosorbent to remove of Cd^{2+} from the wastewater in future. Moreover, previous research had shown that the maximum sorption capacity of immobilized biomass was much higher than that of non-immobilized biomass [34]. Studies concerning immobilized *L. aequinoctialis* and its effect on heavy metal ion adsorption will be carried out in the future.

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