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# Studying effect of cell wall's carboxyl–carboxylate ratio change of *Lemna minor* to remove heavy metals from aqueous solution

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ABSTRACT

The pre-treated *Lemna minor* can remove Hg(II), Cr(III), Cr(VI) and Cu(II) from the aqueous solution. The concentration determination of carboxyl and carboxylate groups and its rule to change the metal ions uptake was done by the curves of the potentiometric titration. It was shown that the removal percent of the heavy metal ions (Co = 1.00 mM) increased 25.1, 26.0, 17.2 and 24.1% for these ions, respectively, with increasing the carboxylate from 0.92 to 2.42 mmol/g *L. minor* and then activating by the activator chloride salts. The removal percent of these ions was decreased 33.1, 27.5, 20.7 and 15.01%, respectively, with increasing the carboxyl from 1.50 to 2.41 mmol/g *L. minor*, inspite of activating by the chloride salts. The enthalpy change ($\Delta H$) was −27.43, −25.94, −28.12 and −22.27 kJ/mol and the entropy change ($\Delta S$) was 81.3, 79.9, 86.1 and 67.7 J/mol K, by activated biomass, respectively. *L. minor* removed these heavy metals corresponding to pseudo-second-order kinetic model that the activation energy ($E_a$) was obtained 18.59, 15.93, 20.36 and 21.12 kJ/mol by the activated and 23.02, 22.27, 23.98 and 24.46 kJ/mol by the reference biomass to uptake Hg(II), Cr(III), Cr(VI) and Cu(II), respectively.



## 1. Introduction

The different methods are used for the removal of heavy metals as important contaminants in water and wastewater. The chemical methods such as the metal ions precipitation with lime or caustic soda, require a large amount of chemicals, which generates volumetric sludge and increases the costs [1,2]. The major advantages of the heavy metals adsorption technology by biomass are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials [3,4]. Furthermore, biosorption methods often provide better results than activated carbon and natural zeolites and are comparable to synthetic ion-exchange resins [5].

Adsorbent materials (biosorbent) derived from suitable biomass can be used for the effective removal and recovery of heavy metal ions from wastewater streams [6]. However, certain types of microbial biomass, even in non-living form, can serve as a basis for development of biosorbent materials for the efficient removal of heavy metals [6,7]. The non-living biosorbents can also be re-used after regenerating the exhausted biomass using a suitable eluant [8]. The biomass includes bacteria [9], fungi [10], yeast [11], marine algae [12] and others.

We had also shown the activated, semi-intact and inactivated *Azolla filiculoides* (an aquatic fern) can remove Pb(II), Cd(II), Ni(II) and Zn(II) [13–16].

The cell wall of plant biomass has proteins, lipids, carbohydrate polymers (cellulose, xylane, mannan, etc.) and inorganic ions of $Ca^{2+}$, $Mg^{2+}$, etc. The carboxylic and phosphate groups in the cell wall are acidic functional groups of biomass and these functional groups direct affect the adsorption capacity of the biomass [17,18].

The initial binding and exchange of heavy metal ions to insoluble constituents in the non-living biomass matrix most probably involves cell wall charged groups. Pectin is an important polysaccharide constituent of plant cell walls, made of fragments of polygalacturonic acid chains with glycosidic bond α (1 → 4), which interact with $Ca^{2+}$ and $Mg^{2+}$ ions to form a three-dimensional polymer [19,20]. The degree of pectin methylation (DM) in the cell wall had been expressed as $[-COOCH_3]/([-COOCH_3]+[-COOH])$ in the chain [21].

The main aim of this work was using potentiometic titration as an analytical method to prove changing –COOH/$–COO^-$ ratio as the effective couple of the cell wall's pectin in *L. minor* (as a plant) by the pre-treatment process and its effect on the heavy metals uptake.

The next objective was to compare the thermodynamic, equilibrium and kinetic parameters between the pre-treated and reference

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**Nomenclature**

| | |
|---|---|
| $A$ | frequency factor |
| ACS | activated *Lemna minor* at the acidic and alkali pHs and with chloride salts |
| $C_o$ | heavy metals initial concentration (mg/l) |
| $C_e$ | heavy metals equilibrium concentration (mg/l) |
| DM | degree of pectin methylation |
| $E_a$ | activation energies (kJ/mol) |
| $k_{1,ads}$ | rate constant of first-order sorption ($min^{-1}$) |
| $k_{2ads}$ | rate constant of second-order biosorption (g/mg min) |
| $K_L$ | Langmuir constant, sorption binding constant ($mg^{-1}$) |
| $m$ | biosorbent dry weight (g) |
| no. ACS | activated *L. minor* at the acidic and alkali pHs, without chloride salts |
| Opt. ACS | activated *L. minor* particles at pH 11 and with chloride salts |
| $q$ | adsorbed heavy metals on the adsorbent at time $t$ (mg/g dry biomass) |
| $q_e$ | adsorbed heavy metals on the adsorbent at equilibrium (mg/g dry biomass) |
| $Q_{max}$ | Langmuir parameter, maximum adsorption capacity (mg/g dry biomass) |
| $R_g$ | universal gas constant (8.314 J/mol K) |
| Reference | *L. minor* as control (washed at pH 7, without chloride salts) |
| $T$ | absolute temperature (K) |
| $V$ | suspension volume (l) |
| $V_{eq}$ | consumed titrant volume to reach the inflection points |

*Greek symbols*

| | |
|---|---|
| $\Delta G$ | free energy change (kJ/mol) |
| $\Delta H$ | enthalpy change (kJ/mol) |
| $\Delta S$ | entropy change (J/mol K) |

*L. minor* with the different and determined values of carboxyl and carboxylate groups to metal ions uptake.

## 2. Materials and methods

### 2.1. L. minor for pre-treatment

The test *L. minor* (a macrophytes biomass) was acquired from a natural lake from the north of Iran. Each 2.0 g of the *L. minor* sample was washed three times with distilled water and was air-dried in sunlight. These obtained *L. minor* samples (as the raw non-living biomass) were then sieved to particles of 2.0 mm before use. The all-experimental solutions in the pre-treatment process were also prepared by distilled water at $22 \pm 2$ °C and agitation rate of 150 rpm. The all pre-treated *L. minor* samples, at last, were dried in oven at 70 °C for 10 h.

### 2.2. Preparing of the pre-treated and reference L. minor

*L. minor* was pre-treated by two following methods: (i) by HCl (0.2 M) at pHs 1, 3, 5 and NaOH (0.2 M) at pHs 9, 11 and 12 with range of ±0.1, as six experiments, separately. This *L. minor* sample was as no. ACS, viz. the obtained biomass without using the activator chloride salts. (ii) At first by HCl and NaOH (0.2 M) at the mentioned pHs, as the first step of pre-treatment and then using

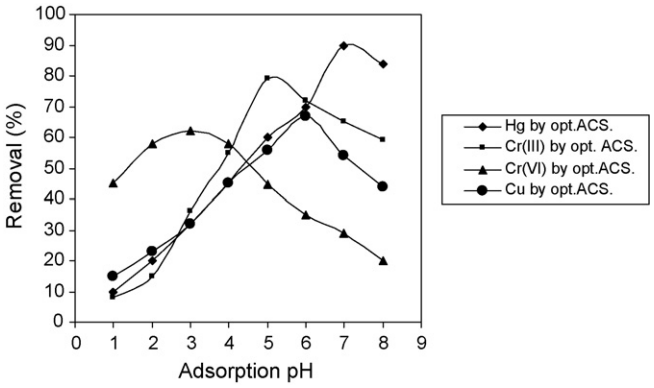


**Fig. 1.** Effect of solution pH (at the adsorption process) on the metal ions removal by activated *Lemna minor* particles at optimal pH and with chloride salts (opt. ACS) at the pre-treatment process. $C_o$ = 1.00 mM, *Lemna minor* dose = 2g/l, biosorption time = 4.0 h.

500 ml of $CaCl_2/MgCl_2/NaCl$ with 1:1:1 molar ratio, as the second step of pre-treatment. This *L. minor* sample was as ACS, viz. the obtained biomass using the activator chloride salts. These chloride salts were used at the total concentrations of 2 M, at pH $7.0 \pm 0.2$ for 5 h.

In order to do these processes, the *L. minor* samples (2.0 g) were soaked in HCl and NaOH solutions (0.2 M) at various pHs for 10 h. The *L. minor* samples were then washed three times with distilled water (each time 100 ml for 0.5 min), to remove excess sodium ions.

The *L. minor* samples which were pre-treated at pH 11 and with chloride salts have the highest ability to remove so are named opt. ACS.

To prepare the reference or control *L. minor*, the biomass samples were soaked in the distilled water with pH $7.0 \pm 0.2$ (in absence of considerable $H^+$ and $OH^-$) for 10 h, without using $CaCl_2/MgCl_2/NaCl$ (viz. no. ACS at pH 7).

The adsorption experiments to obtain the removal data by ACS and no. ACS was done by the mentioned *L. minor* at the optimal obtained pHs (from Fig. 1) for 4.0 h as the confident and sufficient time to remove every metal ion.

### 2.3. Acid–base and base–acid potentiometric titrations

The pre-treated biomass were washed with deionized water, and then the suspensions as 0.2 g of *L. minor* in 100ml of solution were potentiometrically titrated with 0.1 M HCl (for the basic pre-treated biomass) and 0.1 M NaOH (for the acidic pre-treated biomass), separately. The reference sample (pre-treated at pH 7) was titrated by HCl and NaOH, individually.

The pH electrode was calibrated with pH 3.00, 6.00 and 9.00 (with range ± 0.01) buffers just before titrations. The initial pH of solutions were adjusted to $7.0 \pm 0.05$ with 0.01 M HCl and NaOH and the solutions were titrated by a Mettler-Toledo DL 53 titrator (Schwerzenbach, Switzerland) to reach pH 4.3–4.5, which corresponds to $pK_a$ values of carboxyl–carboxylate equilibrium point corresponding. The solutions in the vessel were agitated by a magnetic stirrer (500 rpm) until pH became stable after each titrant aliquot injection. The pH was noted when the value was stable for 100 s.

### 2.4. Batch sorption experiments and equilibrium model

The Hg(II), Cr(III), Cr(VI) and Cu(II) stock solutions were prepared by dissolving their corresponding salts, viz. $HgCl_2$, $CrCl_3 \cdot 3H_2O$,

$K_2Cr_2O_7$, $CuCl_2$ (analytical grade from Merck) in distilled water, separately and standardized by atomic adsorption spectrophotometry.

To avoid the analysis error of Cr(III) due to the partial oxidation of Cr(III) to Cr(VI), the content of the produced Cr(VI) was determined after oxidation all Cr(III) to Cr(VI) using $KMnO_4$ 0.2 M solution, adequately as a powerful oxidant. The Cr(III) concentration in the samples was determined on the basis of analyzing the content of Cr(VI). The excess $KMnO_4$ was removed from biomass by washing with distillated water (multiple), after pre-treatment process.

For equilibrium studies (to obtain adsorption isotherms), a series of flasks (250 ml, as batch sorption reactors) were prepared containing heavy metal solutions (100 ml) of known concentrations ($C_o$) varying from 0.53 to 4.20 mM (each solution contained one metal ion). The experiments conditions were as follows: addition of pre-treated *L. minor* (200 mg) into each flask (dose 2.0 g biomass/l), agitating mixtures (150 rpm) for10 h as the adsorption time at each of used temperatures, viz. 10, 25 and 40 °C with changes range of ±0.5 °C and adjusting adsorption pH at the optimal values for each metal ions (according to Fig. 1) using 0.1 M NaOH and 0.1 M HCl during the equilibrium period. The biomass was removed at last by filtration through a 0.45 μm membrane filter (Millipore) and the filtrate was analysed for ion content ($C_e$) by atomic absorption spectrometry.

The isotherms can be described by Langmuir equation that is suitable for adsorption by non-living biomass [8]:

$$q_e = \frac{Q_{max}\, K_L\, C_e}{1 + K_L C_e} \quad (1)$$

The Langmuir equation transforms to the linearized form:

$$\frac{C_e}{q_e} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max} K_L} \quad (2)$$

that $Q_{max}$ and $K_L$ are found from the slop and intercept of $C_e/q_e$ versus $C_e$ linear plot such that $Q_{max}$ = 1/slope, and $b$ = (slope/intercept). $q_e$ is given from the following relation:

$$q_e = \frac{C_o - C_e}{m/V} \quad (3)$$

where $C_o$ is the initial concentration of the metal ions (mM or mg/l), $m$ is the biosorbent dry weight (g) and $V$ is the suspension volume (l).

### 2.5. Biosorption pH and blank tests

The pH of solutions were adjusted from 1.0 to 8.0, individually, for each metal ion sample ($C_o$ = 1.00 mM) by 0.2 M HCl and 0.2 M NaOH solutions to study the biosorption process. The used *L. minor* samples were those that were treated at pH 11.0 ± 0.2 by 0.2 M NaOH solution in the first step and chloride salts in the second step of pre-treatment process (as opt. ACS). The biomass dose and the contact time were selected 2.0 g/l and 4.0 h (as a confident and sufficient time to remove the metal ions), respectively. The work at the pH values higher than the limits that the metal ions were precipitated get restricted the true biosorption studies. So it was used the 0.2 M HCl solutions to dissolve the precipitates to determine the concentration of the unsorbed metal ions. All the biosorption experiments were run at the obtained optimal pH values from Fig. 1 for each metal ion.

*Blank tests*: In order to eliminate the ions removal effect in the high pHs by the precipitating as the hydroxide salts, the blank tests were carried out. In these tests, were selected the samples of within cations solution, only (without *L. minor*) and separately, as blank in the same conditions of the pre-treatment experiments. The correction was applied in comparison between the removal values in the blank and experimental solutions for the ions at all studies. Therefore we could determine the cations that are removed only by *L. minor* easily.

### 2.6. Biosorption time

The contact times of 25–200 min were selected for the metal solutions ($C_o$ = 1.00 mM) with 2.0 g biomass/l at the obtained optimal pHs for each metal ion from the previous study. These pH values were almost 7.0, 5.0, 3.0 and 6.0 for Hg(II), Cr(III), Cr(VI) and Cu(II), respectively (Fig. 1). The used *L. minor* samples also were those that were treated at pH 11.0 ± 0.2 by 0.2 M NaOH solution and with chloride salts (opt. ACS).

## 3. Results and discussion

### 3.1. Effect of adsorption pH on the heavy metals uptake

Fig. 1 shows that the metal ions uptake increased continuously with increasing pH from 2.0 to 7.0, 5.0 and 6.0 to remove Hg(II), Cr(III) and Cu(II), respectively. As a general rule, the pH influence on metal uptake by algal biomass is closely related to the ionic states of the cell wall functional groups as well as to the metal speciation in solution. The increase in sorption efficiency can be easily correlated to the effect of proton competition on chelation mechanism. At increasing the pH, the competition by proton decreased and so the affinity of the sorbent for metal cations increased.

At lower pH values (<5) carboxyl groups (−COOH) in the plants cell wall retained their protons reducing the possibility of binding to any positively charged ions. Whereas at higher pHs above 5, the carboxylate (–$COO^-$) ligands attract positively charged metal ions and binding occurs, indicating that the major process is an ion exchange mechanism that involve an electrostatic interaction between the positively charged groups in cell walls and metallic cations [22–24]. Uptake capacity of Cr(III) with seaweed biosorbent was reported negligible at lower pH range 1.0 and 2.0 and $Cr(OH)^{2+}$ cations exist in solutions at pH < 2.5, and Cr(III) starts precipitating as $Cr(OH)_3$ at pH > 5.0 [25]. On the other hand, Cu(II) uptake by biomass is decreased after pH 6 due to precipitate $Cu(OH)_2$, which starts precipitating from solutions at higher pH values, making true sorption studies impossible, similar results have been reported for metal biosorption studies in literature [22–24].

Adsorption of Cr(VI) below pH 3.0 (maximum at pH 2.0) suggests that the negatively charged chromium species (chromate/dichromate in the sample solution) bind through electrostatic attraction to positively charged functional groups on the surface of algal cell wall because at this pH more functional groups carrying positive charges would be exposed. But at pH above 3.0, it seems that algal cell wall possesses more functional groups carrying a net negative charge which tends to repulse the anions.

However, there is removal above pH 3.0 also, as is indicated by Fig. 1, but the rate of removal is considerably reduced. Hence, it could be said that above pH 3.0, other mechanism like physical adsorption on the surface of sorbent could have taken an important role in sorbing Cr(VI) and exchange mechanism might have reduced [26].

Therefore, the optimal pH values to remove Hg(II), Cr(III), Cr(VI) and Cu(II) by the dead *L. minor* were 7.0, 5.0, 2.0 and 6.0, respectively, that used for the biosorption processes in this study.

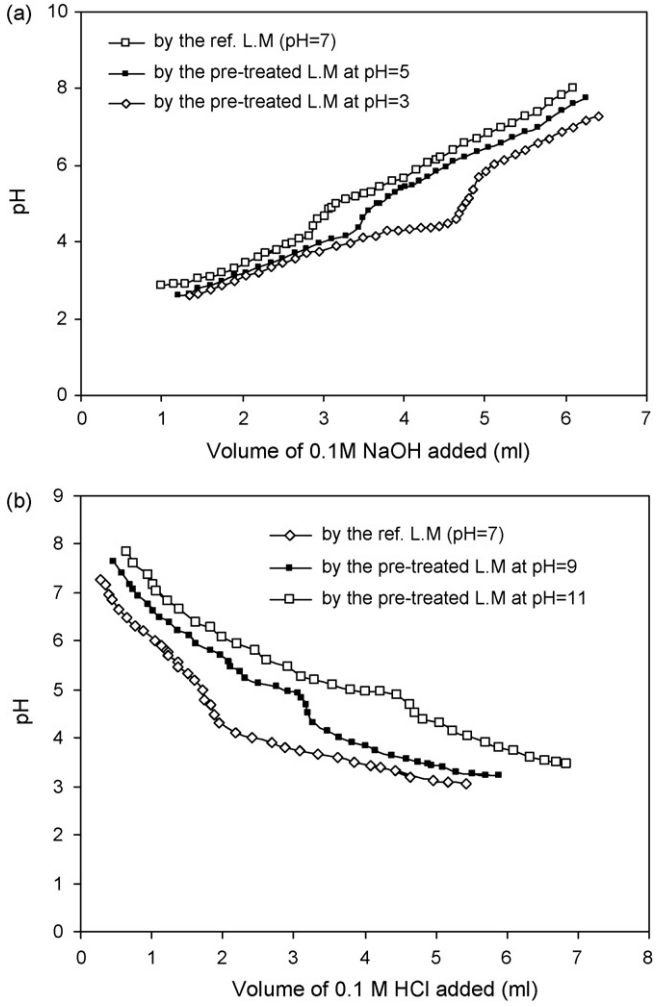


**Fig. 2.** Potentiometric titration curves of the *Lemna minor* with alkali (a) and acidic (b) agents. *Lemna minor* dose = 2 g/l.

### 3.2. Potentiometric titration curves to study the L. minor pre-treatment

In biosorption study, titration is often used to characterize the type of functional groups and their concentrations on the biosorbent surface, which is very important in evaluating its adsorption capacity and mechanisms. Fig. 2a and b shows the acid–base titration curves of the pre-treated biomass.

From the potentiometric titration data, it is possible to make a qualitative and semi-quantitative determination of the nature and number of active sites present on the biomass. The titration curves show the one inflection points at approximately pH 4.3–4.5, which corresponds to p$K_a$ values of carboxyl–carboxylate equilibrium point [27–30]. For pH values greater than the p$K_a$, the sites are mainly in dissociated form and can exchange $H^+$ with metal ions in solution; while at pH values lower than p$K_a$, complexation phenomenon can also occur [31].

The –COOH groups of pectin are converted to $-COO^-$ groups during the alkali titration (with NaOH) and the $-COOCH_3$ groups are converted to –COOH groups during the acidic titration (with HCl). The number of carboxyl (weak acid) and carboxylate (weak base) groups per gram of biomass (mmol/g) can be calculated by the estimation of inflection points ($V_{eq}$, ml) in the titration curves,

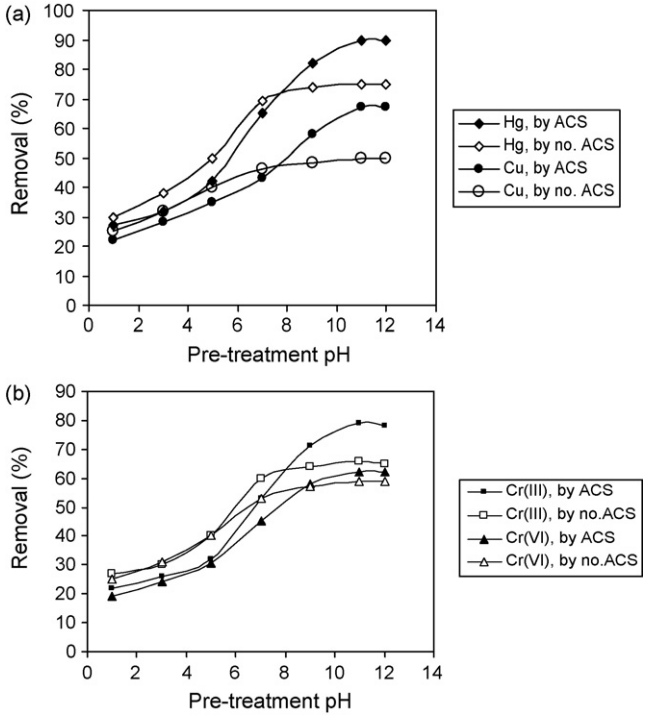


**Fig. 3.** Kinetics of heavy metals uptake by opt. ACS, reference *Lemna minor*, individually. $C_o$ = 1.00 mM, *Lemna minor* dose = 2 g/l, adsorption pH was the optimal values for each ion uptake, $T$ = 25 °C.

using the following equations [30]:

$$[COOH]_{total} = \frac{V_{eq(NaOH)} C_{NaOH}}{m} \quad (4)$$

and

$$[COO^-]_{total} = \frac{V_{eq(HCl)} C_{HCl}}{m} \quad (5)$$

where $m$ (g) was the mass of *L. minor*.

As can be seen from Fig. 3a and b, increasing pre-treatment pH increases the removal values that this is more remarkable after pH 7 by ACS *L. minor*, contrary to no. ACS biomass. It can be due to increase $[COO^-]_{total}$ (according to Table 1) in the first step of pre-treatment, accompanied by using $CaCl_2/MgCl_2/NaCl$ (1:1:1) in the second step of pre-treatment for ACS type.

It can be explained due to $Ca^{2+}$, $Mg^{2+}$ and $Na^+$ replacement instead of the removed ions from *L. minor*. In this study, therefore, the exchanger ions could be lost due to *L. minor* washing by distilled water (pH 7.0 ± 0.05) in the preliminary stage (prior to activation process) and in the pre-treatment processes at the various pHs.

**Table 1**
The changes of the carboxyl and carboxylate values in the *Lemna minor* cell wall due to change the pre-treatment pH

| Pre-treated pH of L.M. | $[COOH]_{total}$ (mmol/g) | $[COO^-]_{total}$ (mmol/g) |
|---|---|---|
| 3 | 2.41 | |
| 5 | 1.81 | |
| 7 (ref.)[a] | 1.50 | 0.92[b] |
| 9 | | 1.61 |
| 11 | | 2.42 |

[a] DM of the ref. *Lemna minor* = $V_{eq(HCl)}/(V_{eq(NaOH)}+V_{eq(HCl)}) = [COOCH_3]/([COOCH_3]+[COOH]) = 0.38$.

[b] It is $[COOCH_3]_{total}$ at pH 7 (the hydrolysis is not done). It is converted to $-COO^-$ at pHs of 9 and 11 by the hydrolysis.

**Table 2**
The change of the removal percent of metal ions ($C_o$ = 1.00 mM) due to the changes of the pre-treatment pH of *Lemna minor* and its relation with the changes of $[COO^-]_{total}$, $[COOCH_3]_{total}$ and $[COOH]_{total}$ as the functional groups of pectin

| Removal changes (%) with the pre-treatment pH changes[a] | | | | |
|---|---|---|---|---|
| | (ΔpH: 7–9)[b] | (ΔpH: 9–11)[c] | (ΔpH: 7–5)[d] | (ΔpH: 5–3)[e] |
| By ACS *Lemna minor* | | | | |
| $Hg^{2+}$ | +17.1 | +8.0 | −22.9 | −10.2 |
| Cr(III) | +18.1 | +7.9 | −21.3 | −6.2 |
| Cr(VI) | +13.2 | +4.0 | −14.4 | −6.3 |
| $Cu^{2+}$ | +15.0 | +9.1 | −7.9 | −7.1 |
| By no. ACS *Lemna minor* | | | | |
| $Hg^{2+}$ | +4.6 | +1.4 | −19.0 | −12.2 |
| Cr(III) | +4.2 | +1.7 | −20.4 | −9.8 |
| Cr(VI) | +4.3 | +2.1 | −13.1 | −9.2 |
| $Cu^{2+}$ | +2.1 | +2.2 | −6.3 | −8.1 |

[a] According to Fig. 3a and b.
[b] According to Table 1, increase of $[COO^-]_{total}$ is 75.0%.
[c] According to Table 1, increase of $[COO^-]_{total}$ is 50.3%.
[d] According to Table 1, decrease of $[COOCH_3]_{total}$ or increase of $[COOH]_{total}$ is 20.6%.
[e] According to Table 1, decrease of $[COOCH_3]_{total}$ or increase of $[COOH]_{total}$ is 33.1%.

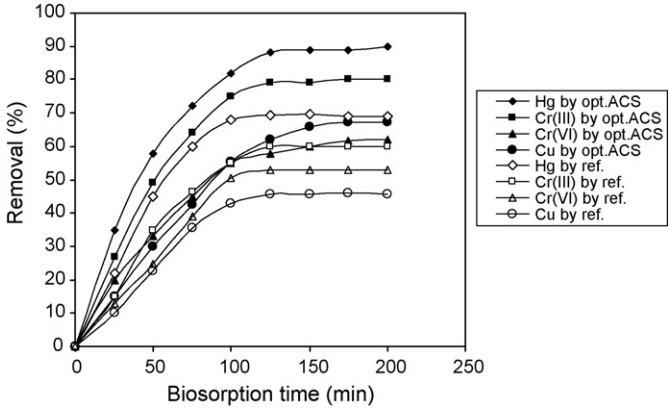


**Fig. 4.** Effect of using NaOH and HCl with (ACS) and without (no. ACS) $CaCl_2/MgCl_2/NaCl$ (molar ratio of 1:1:1, respectively) in *Lemna minor* pre-treatment, respectively, to remove $Hg^{2+}$ and $Cu^{2+}$ (a) and $Cr^{3+}$ and $Cr^{6+}$ (b), $C_o$ = 1.00 mM, *Lemna minor* dose = 2 g/l, adsorption pH was the optimal values for each ion uptake, $T$ = 25 °C, biosorption time = 4.0 h.

On the other hand, the more consumption of HCl (0.2 M) in the first step of pre-treatment process (from pH 7 to 5 and 3), decreased the *L. minor* ability to remove the heavy metals, with and without using $CaCl_2/MgCl_2/NaCl$ (1:1:1) in the second step of pre-treatment (ACS and no. ACS, respectively). It can be due to decrease of $[COOCH_3]_{total}$ or increase of $[COOH]_{total}$ (according to Table 1).

As can be seen from Table 2 increasing $-COO^-$ groups by the pre-treatment pH changes from 7 to 11 and then activating by the chloride salts increases the metal ions uptake, while increasing –COOH by the pre-treatment pH changes from 7 to 3 decreases the metal ions uptake in despite of activating by the chloride salts.

The share of $Ca^{2+}$, $Mg^{2+}$ and $Na^+$ to increase the heavy metal ions uptake can be obtained by comparison of the removal percents by ACS and no. ACS *L. minor*. The removal percents of metal ions by no. ACS *L. minor* ($C_o$ = 1.00 mM) was higher than ACS one at the pre-treatment pHs of before 7.0, but it was higher by ACS biomass than no. ACS one at the pre-treatment pHs of after 7.0. It clears the rule of $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ after increasing –COOH groups to effective ion exchange.

Using $CaCl_2/MgCl_2/NaCl$ can increase $(-COO)_2Ca$ in the pectin structure of cell wall by exchange of each a $Ca^{2+}$ with two $H^+$ of neighbor carboxyl groups [20] and also $(-COO)_2Mg$ bindings by $Mg^{2+}$, similarly. Each $H^+$ can be exchanged with each $Na^+$ and so are increased –COONa groups (or $-COONa_2OOC-$) in the cell wall, however, as mentioned before this, $Na^+$ is mostly collected in biomass cell as soluble salts. These states increase the *L. minor* ability for the ion-exchange or removal of the heavy metals in the adsorption process.

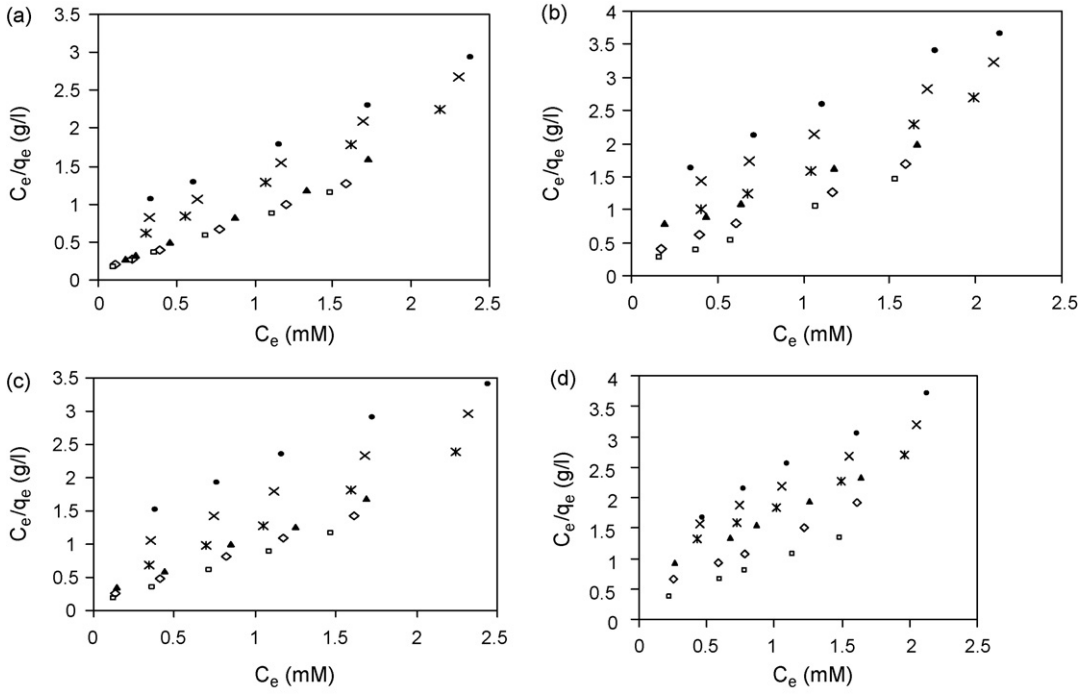


**Fig. 5.** Langmiure isotherms for removal of $Hg^{2+}$ (a), $Cr^{3+}$(b), $Cr^{6+}$(c) and $Cu^{2+}$(d) by opt. ACS *Lemna minor* at 10 °C (□), 25 °C (△), 40 °C (▲) and reference particles at 10 °C (*), 25 °C (×), 40 °C (■). $C_o$ = 1.00 mM, dose = 2 g/l, adsorption pH was the optimal values for each ion uptake, biosorption time = 4.0 h.

**Table 3**
Langmuir and thermodynamic parameters for metal ions adsorption onto the ACS *Lemna minor*, $C_o$ = 1.00 mM, dose = 2g/l, biosorption time = 4.0 h, and adsorption pH was the optimal values for each ion uptake with using temperatures of 283, 298 and 313 K

| | $T$ (K) | Opt. ACS *Lemna minor* | | | | | |
|---|---|---|---|---|---|---|---|
| | | $Q_{max}$[a] (mmol/g) | $K_L$[a] ($mM^{-1}$) | −(G[b] (kJ/mol) | −($H$[b] (kJ/mol) | ($S$[b] (J/mol K) | ($R^2$)[c] |
| | 283 | 1.269 | 6.119 | 4.262 | | | |
| $Hg^{2+}$ | 298 | 1.126 | 4.184 | 3.546 | 27.43 | 81.3 | 0.9548 |
| | 313 | 1.019 | 1.984 | 1.784 | | | |
| | 283 | 1.023 | 3.939 | 3.226 | | | |
| Cr(III) | 298 | 0.968 | 2.554 | 2.324 | 25.94 | 79.9 | 0.9819 |
| | 313 | 0.955 | 1.362 | 0.804 | | | |
| | 283 | 1.218 | 4.698 | 3.848 | | | |
| Cr(VI) | 298 | 1.146 | 2.494 | 2.265 | 28.12 | 86.1 | 0.9852 |
| | 313 | 1.021 | 1.637 | 1.283 | | | |
| | 283 | 1.214 | 3.416 | 3.197 | | | |
| $Cu^{2+}$ | 298 | 1.156 | 2.159 | 1.907 | 22.27 | 67.7 | 0.9793 |
| | 313 | 1.003 | 1.575 | 1.183 | | | |

[a] Obtained from Eq. (2) with $R^2 > 0.98$.
[b] Obtained from Eq. (7) with ($R^2$)[c].

### 3.3. Effect of biosorption time on the heavy metals uptake (kinetic studies)

As shown in Fig. 4, the removal rate of metal ions is arranged as follows: Hg(II) > Cr(III) > Cr(VI) > Cu(II), so that Hg(II) and Cr(III) uptake were completed after almost 50 min, while it was happened for Cr(VI) and Ni(II) uptake after almost 120 and 150 min, respectively. The maximum uptake of Hg(II), Cr(III), Cr(VI) and Cu(II) ($C_o$ = 1.00 mM) after the mentioned contact time by the treated biomass at pH 11.0 ± 0.2 (by 0.2 M NaOH solution) were obtained 72.5, 64.5, 67.6 and 60%, respectively.

### 3.4. Adsorption isotherms in the batch reactors

Fig. 5a–d shows the obtained adsorption isotherms by opt. ACS and reference *L. minor* at three different temperature; 283, 298 and 313 K. According to Tables 3 and 4, $Q_{max}$ and $K_L$ values were decreased to remove heavy metal ions with increasing temperature for both opt. ACS and reference biomass. On the other hand, $Q_{max}$ and $K_L$ values for each metal ion uptake by opt. ACS *L. minor* were greater than those for reference biomass at the same temperature. It also can be seen that the change of biosorption temperature has a more effect on the shift and slop of obtained sorption isotherms by the reference biomass, resulting on their $Q_{max}$ and $K_L$ values.

### 3.5. Thermodynamic study

The free energy change of the sorption reaction is given by

$$\Delta G = -R_g T \ln K_L \quad (6)$$

According to the following equation:

$$\ln K_L = \frac{-\Delta G}{R_g T} = \frac{-\Delta H}{R_g T} + \frac{\Delta S}{R_g} \quad (7)$$

the plot of $\ln K_L$ as a function of $1/T$ (Van't Hoff plots) yields a straight line that $\Delta H$ and $\Delta S$ are found from the slop and intercept, respectively. $R_g$ is the universal gas constant (8.314 J/mol K) and $T$ is the absolute temperature ($K$).

As can be seen from Tables 3 and 4, the negative values of $\Delta H$ confirms the exothermic character of biosorption on mentioned metal ions-*L. minor* (opt. ACS and reference biomass) whereas the low values of $\Delta S$ indicates that no remarkable change on entropy associated to the biosorption process. The negative values of $\Delta G$ validate the feasibility of the sorption process, and the spontaneity

**Table 4**
Langmuir and thermodynamic parameters for metal ions adsorption onto the reference *Lemna minor*, $C_o$ = 1.00 mM, dose = 2g/l, and adsorption pH was the optimal values for each ion uptake with using temperatures of 283, 298 and 313 K

| | $T$ (K) | Opt. ACS *Lemna minor* | | | | | |
|---|---|---|---|---|---|---|---|
| | | $Q_{max}$[a] (mmol/g) | $K_L$[a] ($mM^{-1}$) | −(G[b] (kJ/mol) | −($H$[b] (kJ/mol) | ($S$[b] (J/mol K) | ($R^2$)[c] |
| | 283 | 0.985 | 2.567 | 2.219 | | | |
| $Hg^{2+}$ | 298 | 0.925 | 2.040 | 1.767 | 14.157 | 42.0 | 0.9782 |
| | 313 | 0.736 | 1.438 | 0.946 | | | |
| | 283 | 0.923 | 2.463 | 2.120 | | | |
| Cr(III) | 298 | 0.863 | 1.756 | 1.394 | 15.145 | 46.1 | 0.9992 |
| | 313 | 0.712 | 1.329 | 0.741 | | | |
| | 283 | 0.985 | 2.423 | 2.082 | | | |
| Cr(VI) | 298 | 0.856 | 1.739 | 1.370 | 11.601 | 33.9 | 0.9599 |
| | 313 | 0.711 | 1.513 | 1.077 | | | |
| | 283 | 0.963 | 1.613 | 1.124 | | | |
| $Cu^{2+}$ | 298 | 0.836 | 1.512 | 1.025 | 4.834 | 13.2 | 0.9469 |
| | 313 | 0.632 | 1.323 | 0.728 | | | |

[a] Obtained from Eq. (2) with $R^2 > 0.98$.
[b] Obtained from Eq. (7) with ($R^2$)[c].

**Table 5**
Comparison between adsorption rate constants of the heavy metals, $q_e$ estimated and coefficients of correlation associated to the Lagergren pseudo-first-order and to the pseudo-second-order kinetic models at 283, 298 and 313 K

| | $T$ (K) | Pseudo-first-order kinetic model | | | Pseudo-second-order kinetic model | | | $q_{e,exp}$ (mg/g) |
|---|---|---|---|---|---|---|---|---|
| | | $-k_{1,ads}$ ($\times10^{-3}$) (min$^{-1}$) | $q_{e,cal}$ (mg/g) | $R^2$ | $k_{2,ads}$ ($\times10^{-3}$) (g/mg min) | $q_{e,cal}$ (mg/g) | $R^2$ | |
| $Hg^{2+}$ | 283[a] | 8.11 | 46.35 | 0.798 | 4.51 | 79.01 | 0.995 | 78.23 |
| | 283[b] | 5.06 | 39.63 | 0.889 | 3.15 | 54.12 | 0.996 | 54.23 |
| | 298[a] | 19.35 | 42.55 | 0.801 | 6.33 | 91.96 | 0.999 | 90.01 |
| | 298[b] | 16.44 | 34.42 | 0.921 | 4.98 | 63.86 | 0.999 | 62.22 |
| | 313[a] | 21.36 | 33.59 | 0.856 | 9.65 | 93.45 | 0.997 | 94.01 |
| | 313[b] | 17.66 | 24.96 | 0.896 | 8.02 | 66.95 | 0.998 | 66.89 |
| $Cr^{3+}$ | 283[a] | 11.52 | 15.65 | 0.754 | 8.65 | 15.52 | 0.999 | 15.23 |
| | 283[b] | 5.85 | 13.96 | 0.799 | 5.51 | 11.34 | 0.998 | 11.02 |
| | 298[a] | 23.78 | 12.53 | 0.846 | 12.04 | 21.01 | 0.998 | 20.81 |
| | 298[b] | 12.31 | 10.96 | 0.886 | 8.36 | 16.20 | 0.999 | 15.63 |
| | 313[a] | 25.45 | 7.09 | 0.942 | 16.57 | 23.12 | 0.996 | 22.96 |
| | 313[b] | 16.54 | 3.96 | 0.985 | 13.63 | 18.32 | 0.995 | 18.04 |
| $Cr^{6+}$ | 283[a] | 9.23 | 14.35 | 0.921 | 11.10 | 17.32 | 0.996 | 17.21 |
| | 283[b] | 4.52 | 10.21 | 0.963 | 5.51 | 10.23 | 0.998 | 10.05 |
| | 298[a] | 16.33 | 11.32 | 0.822 | 17.39 | 21.31 | 0.999 | 20.96 |
| | 298[b] | 11.41 | 8.02 | 0.952 | 8.59 | 14.06 | 0.998 | 13.07 |
| | 313[a] | 18.63 | 7.02 | 0.855 | 25.32 | 24.52 | 0.999 | 23.96 |
| | 313[b] | 14.32 | 4.21 | 0.796 | 14.63 | 15.96 | 0.998 | 15.55 |
| $Cu^{2+}$ | 283[a] | 8.21 | 13.52 | 0.896 | 11.56 | 18.65 | 0.998 | 18.23 |
| | 283[b] | 6.21 | 9.29 | 0.963 | 4.47 | 11.58 | 0.997 | 11.02 |
| | 298[a] | 20.24 | 10.60 | 0.826 | 18.89 | 23.55 | 0.998 | 22.88 |
| | 298[b] | 14.28 | 6.03 | 0.936 | 6.96 | 15.42 | 0.999 | 14.71 |
| | 313[a] | 23.21 | 6.39 | 0.756 | 27.32 | 27.78 | 0.999 | 26.96 |
| | 313[b] | 17.63 | 4.20 | 0.823 | 12.11 | 17.63 | 0.998 | 17.07 |

To avoid showing the similar figures, the diagram contact time has only showed for 298K, in Fig. 4.
[a] Opt. ACS *Lmna minor*.
[b] Reference *Lmna minor*.

of sorption. The biosorption process of these metal ions by activated *L. minor* was more spontaneity and exothermic than those for the reference or non-activated biomass.

### 3.6. Kinetic modeling

There have been several reports [32,33] on the use of different kinetic models to adjust the experimental data of heavy metals adsorption on biomass. With respect to the kinetic modeling, the first- and second-order kinetic models have been used.

The first-order rate expression of Lagergren is that considers that the rate of occupation of adsorption sites is proportional to the number of unoccupied sites. The linearized form of the pseudo-first-order model is written as

$$\log(q_e - q) = \log q_e - \left(\frac{k_{1,ads}}{2.303}\right) t \quad (8)$$

where $q_e$ and $q$ (mg/g) are the amount of adsorbed heavy metals on the adsorbent at equilibrium and at time $t$ (min) and $k_{1,ads}$ (min$^{-1}$) is the rate constant of first-order sorption. Linear plots of $\log(q_e - q)$ versus $t$ indicate the applicability of this kinetic model [32]. However, to adjust Eq. (8) to the experimental data, the value of $q_e$ (equilibrium sorption capacity) must be preestimated by extrapolating the experimental data to $t = \infty$.

The Lagergren first-order rate constant ($k_{1,ads}$) and the equilibrium amount of metal removed ($q_e$) determined from the model are presented in Table 3 along with the corresponding correlation coefficient. However, the most important feature of this model is that it fails to estimate $q_e$. The linearized form of the pseudo-second-order model is written as

$$\frac{t}{q} = \frac{1}{k_{2,ads} q_e^2} + \left(\frac{1}{q_e}\right) t \quad (9)$$

where $k_{2,ads}$ (g/mg min) is the rate constant of second-order biosorption. The plot $t/q$ versus $t$ should give a straight line if second-order kinetics are applicable and $q_e$ and $k_{2,ads}$ can be determined from the slope and intercept of the plot, respectively. It is important to notice that for the application of this model the experimental estimation of $q_e$ is not necessary. Both parameters and the correspondent coefficients of correlation are also presented in Table 5. The correlation coefficients for the second-order kinetic model are equal to 0.998 and 0.999 for metal ions uptake by opt. ACS. and reference biomass and the theoretical values of $q_e$ also agree very well with the experimental ones. Both facts suggest that the sorption of these heavy metal ions followthe second-order kinetic models, which relies on the assumption that biosorption may be the rate limiting step. As can be seen from Table 5, according to the second-order kinetic model, the adsorbents with due attention to their $k_{2,ads}$ values to remove each heavy metal by opt. ACS was higher than it by reference *L. minor*.

### 3.7. Activation energies comparison of metal ions uptake

Activation energy is determined according to the Arrhenius equation:

$$\ln k = \frac{-E_a}{R_g T} + \ln A \quad (10)$$

where $k$ is the rate constant according to the order of the fitted kinetic model for experiments (in this study is $k_{2,ads}$), $E_a$ is the activation energy, $T$ is the temperature in Kelvin, $R_g$ is the gas constant

**Table 6**
Arrhenius equations and activation energies of metal ions uptake by opt. ACS *Lmna minor* and the reference *Lmna minor*

| | Arrhenius model | $E_a$ (kJ/mol) | $A$ | $R^2$ |
|---|---|---|---|---|
| $Hg^{2+}$ | $y = -2236.1x + 2.4813$[a] | 18.59 | 11.95 | 0.9920 |
| | $y = -2769.6x + 4.0124$[b] | 23.02 | 55.27 | 0.9983 |
| $Cr^{3+}$ | $y = -1916.3x + 2.0167$[a] | 15.93 | 7.51 | 0.9996 |
| | $y = -2678.7x + 4.2454$[b] | 22.27 | 69.78 | 0.9946 |
| $Cr^{6+}$ | $y = -2449.8x + 4.1595$[a] | 20.36 | 64.03 | 0.9996 |
| | $y = -2884.5x + 4.9705$[b] | 23.98 | 144.09 | 0.9942 |
| $Cu^{2+}$ | $y = -2540.7x + 4.5283$[a] | 21.12 | 92.60 | 0.9974 |
| | $y = -2942.5x + 4.9619$[b] | 24.46 | 142.86 | 0.9925 |

[a] Opt. ACS *Lmna minor*.
[b] Reference *Lmna minor*.

(8.314 J/mol K) and $A$ is the constant called the frequency factor. Value of $E_a$ can be determined from the slope of $\ln k$ versus $1/T$ plot (Table 6). The magnitude of activation energy may give an idea about the type of sorption. Chemical adsorption is specific and involves forces much stronger than in physical adsorption. The activation energy for chemical adsorption is of the same magnitude as the heat of chemical reactions. Two kinds of chemical adsorption are encountered, activated and, less frequently, non-activated. Activated chemical adsorption means that the rate varies with temperature according to a finite activation energy (between 8.4 and 83.7 kJ/mol) in the Arrhenius equation (high $E_a$). However, in some systems chemisorption occurs very rapidly, suggesting the activation energy is near zero. This is termed non-activated chemisorption [34].

## 4. Conclusion

On the base of the experimental results of this investigation, the following conclusion can be drawn:

1. Potentiometric titration can be useful to study the pre-treatment process of biomass (*L. minor*) using the acidic and alkali agents. It is done by determining the concentration of carboxyl and carboxylate, as the effective functional groups to the metal ions uptake.
2. The $Q_{max}$ and $K_L$ values (Langmuir constants) to remove Hg(II), Cr(III), Cr(VI) and Cu(II) from the aqueous solution by the activated *L. minor* at the alkali solution and by $CaCl_2/MgCl_2/NaCl$ with 1:1:1 molar ratio was higher than those for the reference one at the same conditions.
3. It was observed that increasing $-COO^-$ groups by the pre-treatment pH changes from 7 to 11 and then activating by the chloride salts increases the metal ions uptake, while increasing –COOH by the pre-treatment pH changes from 7 to 3 decreases the metal ions uptake in despite of activating by the chloride salts.
4. The removal percents of metal ions by no. ACS *L. minor* ($C_o$ = 1.0 mM) was higher than ACS one at the pre-treatment pHs of before 7.0, but it was higher by ACS biomass than no. ACS one at the pre-treatment pHs of after 7.0. It clears the rule of $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ after increasing –COOH groups to effective ion exchange.
5. The thermodynamic studies showed that the biosorption process of these metal ions by activated *L. minor* was more spontaneity and exothermic than those for reference biomass.
6. The kinetic modeling showed that only the data corresponding to the first 25–30 min are adjusted approximately with pseudo-first-order model, since after this period the experimental data deviated considerably from those theoretical. While these data were fitted well with pseudo-second-order kinetic model and uptake activation energies ($E_a$) was less for activated biomass.

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