Statistical Design of Experiments as a Tool for Optimizing the Biosorption of Pb$^{2+}$ and Cd$^{2+}$ on Eichhornia crassipes (Mart.) Solms

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Abstract: *Eichhornia crassipes* approaches being a scourge in many parts of the world, choking waterways and hindering transport upon them. At the same time it is known to readily absorb heavy metal ions from water and, thus, aids in the removal of heavy metals found in such waters. This research considers the possibility of using an experimental design technique to investigate the biosorption of cadmium and lead ions from water solutions, simulating typical industrial effluents. The removal of Cd$^{2+}$ and Pb$^{2+}$ was studied, separately, using the factorial design$^2$. The three factors considered were pH (2.0 and 6.0), T (20 and 45°C) and metal ion concentration (10 and 1200 mg l$^{-1}$). Experiments were carried out in a batch type reactor system with 0.2 g of biosorbent (dead dried biomass of *E. crassipes*) and 50 ml of Cd$^{2+}$ and Pb$^{2+}$ solutions. The removal efficiencies of both ions during an exposition time of 6 h were then evaluated. The results were analyzed statistically using analysis of variance to define the most important process variables affecting the metal removal efficiency. The most significant effect for Cd$^{2+}$ and Pb$^{2+}$ biosorption was ascribed to pH. The interaction effects of XpH and TpH also have a significant influence on the Cd$^{2+}$ and Pb$^{2+}$ removal efficiency, respectively. A normal distribution was observed between the predicted values (model) and the observed (experimental).

Key words: *Eichhornia crassipes* • Biosorption • Factorial experimental design

INTRODUCTION

The release of different pollutants into surface and ground water has increased noticeably as a result of industrialization and thereby lowered the quality of the environment to alarming levels [1]. Of such pollutants, heavy and toxic metals are the most important because of their non-biodegradability, with lead and cadmium ions being the toxic and hazardous [2]. Lead has special industrial significance since it is employed in batteries, paints, plastics, glass and metal industries [3]. Moreover, its contamination is also due to vehicular traffic and the mixing of road side run-offs [4]. On the other hand, cadmium toxicity may be observed by a variety of syndromes and effects including renal dysfunction, hepatic injury and lung damage [5]. Over a few decades, numerous processes such as chemical precipitation, reverse osmosis and solvent extraction have been used for the removal of heavy metal ions from aqueous solution [6]. However, these techniques have certain disadvantages such as incomplete metal removal, high reagent and energy requirements and generation of toxic sludge that require disposal. However, to attain the toxic specie residual concentration in the effluent with acceptable contents according to the legislation, other operations are required.

Due to the high costs of commercial adsorbents, the search for alternate and innovative treatment techniques has focused attention on the use of biological materials for heavy metal removal and recovery technologies (Biosorption). This technique has gained important credibility during recent years due to its effectiveness in reducing the high concentration of heavy metal ions (from industrial wastewater) to very low levels. It is considered a potentially viable method on both technical and economic grounds, because of its low operating costs [7] and the decontamination efficiency for highly diluted effluents. Additionally, metal can be recovered from the biosorbent and reused. Different types of biomass have been investigated for the biosorption characteristics of Pb$^{2+}$ and Cd$^{2+}$ from aqueous solution [8]. The seaweed *Sargassum* sp was used [9] for removal of lead and
cadmium ions from water. Moreover, [10] studied the removal of Cu\(^{2+}\), Pb\(^{2+}\) and Cd\(^{2+}\) ions by biosorption on bacterial cells. Also, [11] used the aquatic plants for removal of lead and zinc ions from waste water. The adsorption of lead ions on nonliving Penicillium chrysogenum biomass was also investigated [12].

Among the biological materials, Eichhornia crassipes (Mart.) Solms (widely distributed aquatic macrophyte) has been reported to have high metal binding capacities and promising results with regard to metal removal from wastewater [13]. It was reported that the ability of E. crassipes to accumulate metal ions was found to be in the order of Pb\(^{2+}\) > Cd\(^{2+}\) > Cu\(^{2+}\) > Zn\(^{2+}\) [14]. The adsorption capacity of Pb\(^{2+}\) affected by experimental parameters such as pH, contact time and concentration of Pb\(^{2+}\) solution, on to E. crassipes plant biomass was studied [15]. They found that the uptake percent of Pb\(^{2+}\) increased by increasing pH values.

However, these authors have evaluated removal efficiencies of heavy metals by this species as a function of one-factor-at-a-time. Few studies employed the factorial design method for evaluating the influence of the operation variables on biosorption processes. The biosorption of Cd\(^{2+}\) and Pb\(^{2+}\) was optimized using 2\(^3\) factorial designs by [16, 17]. The biosorption of Cr\(^{6+}\) and Cr\(^{4+}\) using 2\(^2\) and 2\(^1\) factorial designs, respectively was studied [18, 19]. Factorial design is employed to define the most important process variables affecting the metal removal efficiency [20]. It is also used to reduce the total number of experiments in order to achieve the best overall optimization of the system [21]. The factorial experimental design methodology involves changing all variables from one experiment to the next. The design determines which factors have important effects on the response as well as how the effect of one factor varies with the level of the other factors. The determination of factor interactions could only be attained using statistical designs of experiments [21], since it cannot be shown when the system optimization is carried out by varying just one factor at the time and fixing the other.

The objective of this study was to establish how pH, temperature and initial concentration of lead and cadmium ions interacted and ultimately affected their removal efficiency from aqueous solutions by means of Eichhornia crassipes biomass. A factorial design 2\(^3\) scheme was used to study the removal of Cd\(^{2+}\) and Pb\(^{2+}\), separately, for the benefit of both the remediation of heavy metal pollutants from aquatic environment and the management of Eichhornia crassipes harvested from wetlands.

**MATERIALS AND METHODS**

**Biomass preparation:** A biomass of E. crassipes was used as biosorbent for the biosorption of Cd\(^{2+}\) and Pb\(^{2+}\). Samples of the biomass were collected from El-Mahmoudiah Canal, branched from the Nile River. They were washed several times using de-ionized water to remove extraneous salts, then dried in an oven at 60°C for 48 h, chopped and sieved. The particles with an average of 0.5 mm were used for the experiments.

**Reagents and equipments:** Doubly distilled water was throughout employed. Initial solutions with different concentrations of Cd\(^{2+}\) and Pb\(^{2+}\) were prepared by proper dilution from stock standards (1000 g l\(^{-1}\) Cd\(^{2+}\) and Pb\(^{2+}\)). The pH adjustment of the solutions were made with aliquots of 1.0 mol l\(^{-1}\) of HNO\(_3\), utilizing a pH/mV handheld meter (Crispin pH meter, pH 25).

**Batch biosorption procedure:** Batch experiments were carried out under the following conditions: 0.2 g of Eichhornia crassipes biomass, 50 ml of Cd\(^{2+}\) and Pb\(^{2+}\) solution and an agitation speed of 200 rpm. The pH, temperature and initial Cd\(^{2+}\) and Pb\(^{2+}\) concentration employed are shown in Table 1. The experiments were carried out with the values of pH (2, 6) that were not influenced by the metal precipitation, as metal hydroxide [22]. The maximum temperature employed in the present study was 45°C, as the higher temperature damages the active sites in the biomass [23]. Samples were collected after 6 hours to reach equilibrium for the sorption system [18]. Control samples were made in absence of any metal. Aliquots for analysis were filtered using glass filter provided with Whatman filter paper and the residual Cd\(^{2+}\) and Pb\(^{2+}\) concentration was measured by Varian ICP-AES.

Sixteen duplicate experiments were carried out: eight for Cd\(^{2+}\) and eight for Pb\(^{2+}\). All possible combinations of variables, called factors in the jargon, were used and a matrix was established according to their high and low levels, represented by +1 and -1, respectively.

The removal efficiency \(R\) of Cd\(^{2+}\) and Pb\(^{2+}\) from aqueous solution was defined as:

\[
R = \frac{C - C_f}{C} \times 100
\]

Where: \(C\) and \(C_f\) are, the initial and final concentrations of Cd\(^{2+}\) and Pb\(^{2+}\), respectively.

**Statistical design of experiments (full factorial design):** For studying the Cd\(^{2+}\) and Pb\(^{2+}\) biosorption on E. crassipes biomass, the removal efficiency \(R\) could
Table 1: High and low levels of factors

<table>
<thead>
<tr>
<th>Element</th>
<th>Cd\textsuperscript{2+}</th>
<th>Pb\textsuperscript{2+}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low level</td>
<td>High level</td>
</tr>
<tr>
<td>T (°C)</td>
<td>20.0</td>
<td>45.0</td>
</tr>
<tr>
<td>X (mg l\textsuperscript{-1})</td>
<td>10.0</td>
<td>1200.0</td>
</tr>
<tr>
<td>pH</td>
<td>2.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* T: Temperature; X: Initial concentration

Table 2: Experimental factorial design results for Cd\textsuperscript{2+} and Pb\textsuperscript{2+} biosorption

<table>
<thead>
<tr>
<th>Factor</th>
<th>Cd\textsuperscript{2+}</th>
<th>Pb\textsuperscript{2+}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Removal efficiency (%)\textsuperscript{*}</td>
<td>Average</td>
</tr>
<tr>
<td>T X pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 1 1</td>
<td>37.6</td>
<td>37.6</td>
</tr>
<tr>
<td>1 -1 1</td>
<td>19.5</td>
<td>22.9</td>
</tr>
<tr>
<td>1 -1 -1</td>
<td>50.0</td>
<td>60.0</td>
</tr>
<tr>
<td>-1 1 1</td>
<td>10.0</td>
<td>15.0</td>
</tr>
<tr>
<td>-1 -1 1</td>
<td>34.8</td>
<td>36.3</td>
</tr>
<tr>
<td>-1 -1 -1</td>
<td>24.5</td>
<td>27.0</td>
</tr>
<tr>
<td>-1 -1 -1</td>
<td>90.0</td>
<td>85.0</td>
</tr>
<tr>
<td>-1 -1 -1</td>
<td>20.0</td>
<td>25.0</td>
</tr>
</tbody>
</table>

* Experiment in duplicate

Table 2 shows the removal efficiency of Cd\textsuperscript{2+} and Pb\textsuperscript{2+} at different levels of factors T, X, and pH. The data were analyzed using the Minitab Statistical Software (release 14.1) to obtain the effects, coefficients, standard deviation of coefficients, and other statistical parameters of the final model.

RESULTS AND DISCUSSION

Metallic ion uptake by a biosorbent in a batch system usually depends on several factors, such as acidity of the medium (pH), initial metallic ion concentration (X), and temperature (T). Other variables such as biosorbent concentration and speed of agitation were kept constant. A full 2\textsuperscript{4} factorial design was used to determine the removal efficiency. The results are presented in Table 2. For treatment of data, the Minitab Statistical Software (release 14.1) was employed in order to obtain the effects, coefficients, standard deviation of coefficients, and other statistical parameters of the final model.

The codified mathematical model employed for the 2\textsuperscript{4} factorial design is:

\[ R = A_0 + A_T T + A_X X + A_pH pH + A_{TX} TX + A_{pH} pH + A_{pH} pH + A_{TX} pH \]  \hspace{1cm} (2)

Where \( A_0 \) represents the global mean and \( A_\) the other regression coefficients.

Substituting the coefficients \( A_\) in Equation (2) by their values from Tables 3 & 5 we get the following equations:

\[ R_{Cd^{2+}} = 41.36 - 8.64 T - 2.94 X + 14.94 pH + 2.06 TX - 10.06 pH + 0.40 X pH + 0.39 TX pH \]  \hspace{1cm} (3)

\[ R_{Pb^{2+}} = 146.52 - 0.07 T - 1.25 X - 21.03 pH + 0.007 X + 0.027 pH + 0.024 X pH + 0.001 T X pH \]  \hspace{1cm} (4)

The effects of the main factors (T, X, pH) represent deviations of the average between high and low levels for each one of them. In case of Cd\textsuperscript{2+}, a change in pH value from low to high level results in 29.87 % increase in the removal efficiency (Table 3). If a variation from high to low is made for T and C, increases of 17.28% and 5.88% in the removal efficiency are observed, respectively. In case of Pb\textsuperscript{2+}, T, X and pH exert an influence in their low levels, increasing removal efficiency by 0.15, 2.50 and 42.06%, respectively. It can be concluded that when the effect of a factor is positive an increase in the value of the removal efficiency is observed when the factor changes from low to high level. In contrast, if the effect is negative, a reduction in removal efficiency occurs for the high level of the same factor.
Analyzing the graphs of Fig. 1A and the values of Table 3, it can be inferred that the pH was the most important variable of the overall biosorption procedure. The positive value of its coefficient meant that the Cd\(^{2+}\) uptake by *E. crusipes* biomass was favored at high pH values (pH 6.0). In order to avoid a disruption of the *E. crusipes* biomass at pH lower than 6.0, this value was fixed for continuing the optimization of this work. The second important factor for overall optimization of the batch system was the interaction of two factors X.pH which was more significant than the main factors T and X. Only the achievement of this result emphasizes the merit of using the traditional design of experiments over the conventional univariate process of optimization of the system. This information would not be acquired in a univariate optimization in biosorption system. The negative value of X.pH coefficient meant that low metal ion concentration with low pH value would lead to an unexplained increase in the removal efficiency of Cd\(^{2+}\) that could not be explained using the univariate procedure of optimization of the system. Otherwise, if the system were being optimized by using univariate procedure, a small dimension of the pH of the solution associated with a small dimension of X could lead to a misinterpretation of the results achieved. The third important factor affect the overall optimization of the batch system was the metallic ion concentration (X). The negative coefficient value justifies that low metallic ion concentration led to high removal efficiency of Cd\(^{2+}\) ions. In Table 4 is presented the analysis of variance for the factorial design 2\(^{3}\) without the insignificant three-way interactions. As can be seen, the main factors and two-way interactions were significant at 5\% of probability level (p < 0.05), as discussed above.

Likewise, the results of Pb\(^{2+}\) biosorption (Table 5 and Fig. 3) demonstrated that the pH was the most important variable. However, the negative value of its coefficient meant that the Pb\(^{2+}\) uptake by *E. crusipes* biomass was favored at low pH values (pH 2.0). Accordingly, the pH is proved to be a key condition affecting adsorption performance of the studied metals. This is in line with
Fig. 1: (A) Cd\(^{2+}\) Normal probability plot of standardized effect at \(p=0.05\). The line at 50% divides the negative effects from the positive ones.
(B) Pareto plot of standardized effect (absolute value) at \(p=0.05\)

Fig. 2: Normal probability plot for the removal efficiency of Cd\(^{2+}\)
Table 5: Statistical parameters for 2^3 design (Pb+)  

<table>
<thead>
<tr>
<th>Term</th>
<th>Effect</th>
<th>Coefficient</th>
<th>S.E. of coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.016</td>
<td>146.52</td>
<td>4.30620</td>
<td>0.029</td>
</tr>
<tr>
<td>Main factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>-0.15</td>
<td>-0.07</td>
<td>0.004</td>
<td>0.055</td>
</tr>
<tr>
<td>X</td>
<td>-2.50</td>
<td>-1.25</td>
<td>0.124</td>
<td>0.063</td>
</tr>
<tr>
<td>pH</td>
<td>-42.06</td>
<td>-21.03</td>
<td>0.936</td>
<td>0.021</td>
</tr>
<tr>
<td>Interaction of two factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.X</td>
<td>0.03</td>
<td>0.01</td>
<td>0.001</td>
<td>0.869</td>
</tr>
<tr>
<td>T.pH</td>
<td>0.03</td>
<td>0.02</td>
<td>0.001</td>
<td>0.031</td>
</tr>
<tr>
<td>X.pH</td>
<td>0.48</td>
<td>0.24</td>
<td>0.025</td>
<td>0.067</td>
</tr>
<tr>
<td>Interaction of three factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.X.pH</td>
<td>0.02</td>
<td>0.01</td>
<td>0.001</td>
<td>0.932</td>
</tr>
</tbody>
</table>

The effects and coefficients are given in coded units. P: probability and S.E.: standard error of coefficient

Table 6: Analysis of variance for removal efficiency of Pb+ - full 2^3 factorial design (coded units)  

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main Effects</td>
<td>3</td>
<td>757.19</td>
<td>2962.82</td>
<td>987.61</td>
<td>309.81</td>
<td>0.042</td>
</tr>
<tr>
<td>2-Way Interactions</td>
<td>3</td>
<td>3159.66</td>
<td>3159.66</td>
<td>1053.22</td>
<td>330.39</td>
<td>0.040</td>
</tr>
<tr>
<td>Residual Error</td>
<td>1</td>
<td>3.19</td>
<td>3.19</td>
<td>3.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3920.04</td>
<td>3.19</td>
<td>3.19</td>
<td>3.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d.f.: degree of freedom. Seq SS: sequential sum of squares, Adj. MS: adjusted sum of squares, F = factor F and p: probability

Many studies concerning sorption of heavy metals by different biomaterials indicated that pH and temperature influence removal efficiency. It was found that the pH is one of the most important environmental factors in biosorption of heavy metal ions [27]. The pH value of solution strongly influences not only the site dissociation of the biomass surface, but also the solution chemistry of the heavy metals: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation, the speciation and the biosorption availability of heavy metals. It was demonstrated that the suitable pH ranges for the various metal ions were slightly different [28]. The results of the present research indicated that the highest removal efficiency for Cd²⁺ was attended at the higher pH value (pH=6) while that for Pb²⁺ was recorded at the lower value (pH=2). These results were concomitant with the findings of [29]. They studied the effect of pH upon heavy metal adsorption by reed biomass in a wide range of pH and concluded that the maximum sorption was observed near neutral condition (pH = 6) for Cd²⁺, Ni²⁺, Cd³⁺ and Zn²⁺, while that for Pb²⁺ was from the acidic range (pH 2-4). The adsorption of Pb²⁺ at lower pH was also observed in other biomaterials such as the biomass Zoogloea ramigera [30] and fungus Mucor rouxii [31]. On the other hand, [15] studied the adsorption capacity of Pb²⁺ affected by experimental parameters such as pH, contact time and concentration of Pb²⁺ solution, on to Eichhornia spectosa plant biomass. They found that the uptake percent of Pb⁺ increased by increasing pH values. Moreover, [24] reported that Pb⁺ removal by different organs of Hemidmsus indicus was unaffected by pH change.

At lower pH, the adsorption of many heavy metals usually took place with low removal efficiency. This occurred because there was a high concentration of proton in the solution and this proton competed with the metal ions informing a bond with active sites on the surface the biomaterials. These bonded active sites thereafter became saturated and was inaccessible to other cations [32, 22]. The biosorption characteristics of Cd²⁺ions from aqueous solution using the green alga (Ulua lactuca) biomass were investigated as a function of pH, biomass dosage, contact time and temperature [26]. They found that the maximum biosorption of Cd²⁺ ions was found at pH 5, 20°C, 60 min and 20 mg l⁻¹ of biosorbent.

Temperature has also an influence on the biosorption of metal ions, but to a limited extent under a certain range of temperature, which indicates that ion exchange mechanism exists in biosorption to some extent [33]. In the present investigation, temperature has no significant

[15, 24-26]. The second important factor for overall optimization of Pb²⁺ biosorption was the temperature (T). Similarly, the negative value of its coefficient justifies that low temperature led to highest biosorption of Pb²⁺. In Fig. 2 B, though the interaction of two factors T.pH was significant, it acquired the least effect on the removal efficiency of Pb²⁺ compared to others. The positive coefficient value of this interaction tells us that both factors should be increased in order to achieve the highest response, contrary to each factor alone. The analysis of variance (Table 6) for the factorial design 2³, without the insignificant three-way interactions, indicated that the main factors and two-way interactions were significant at 5% of probability level (p < 0.05).

Optimal conditions realized from the optimization experiment (observed values) were verified by comparing with calculated data from the model (predicted values). Figs. 2 and 4 present the normal probability plot of predicted removal efficiency for Cd²⁺ and Pb²⁺, respectively. In both cases, it was observed how closely the set of observed values with the predicted ones, with correlation coefficients (R) of 0.971 and 0.993 for Cd²⁺ and Pb²⁺, respectively.
Fig. 3: (A) Pb$^{2+}$ Normal probability plot of standardized effect at $p=0.05$. The line at 50% divides the negative effects from the positive ones.
(B) Pareto plot of standardized effect (absolute value) at $p=0.05$

Fig. 4: Normal probability plot for the removal efficiency of Pb$^{2+}$
effect on the biosorption efficiency of Cd$^{2+}$ onto E. crassipes biomass. It was found that temperature (5-40°C) had minor effect on the sorption level of Cd, Cu or Co by Saccharomyces cerevisiae [34]. However, higher removal efficiency of Pb$^{2+}$ was detected at low temperature condition (20°C). This revealed the exothermic nature of Pb$^{2+}$ biosorption onto the studied plant biomass [35]. The adsorption of Cd$^{2+}$ and Pb$^{2+}$ onto carboxymethylated lignin from sugarcane bagasse and Ulva lactuca biomass, respectively, was studied [16, 33]. They reported that the decrease in the biosorption of both ions with the rise in temperature may be due to either the damage of active binding sites in the biomass [23] or increasing tendency to desorb metal ions from the interface to the solution [36].

In the present research, the metal ion concentration has no effect on the biosorption efficiency of Pb$^{2+}$ onto E. crassipes biomass. However, the results showed that the biosorption of Cd$^{2+}$ increased by decreasing its initial concentration in the solution (10 mg L$^{-1}$). The sorption of Cd$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ by Ca-treated Sargassum sp. biomass was compared under low and high ionic strength conditions and an exponential decrease in the removal efficiency of the sorption system with increasing metal concentration was reported [37].

Since there is no literature report on the adsorption of heavy metals by E. crassipes biomass using factorial design, the results obtained were compared with those of many different types of biomaterials. The effects of pH (4.0 and 5.5), initial metal concentration (5.0 and 10.0 g L$^{-1}$) and biomass concentration (0.4 and 0.7 g L$^{-1}$) on biosorption of Cd$^{2+}$ using Aspergillus niger was studied [17]. The biosorption process studied was modeled based on 2$^3$ factorial designs. The most important factor was the biomass concentration. An increase in the removal efficiency occurred with an increase in biomass concentration and pH. However, the removal efficiency decreased with an increase in initial metal concentration. Although the biosorbent mass was constant in the present experiment, pH showed the same tendency in both cases. Moreover, the interaction effects X.pH have significant influence on Cd$^{2+}$ removal efficiency. The biosorption of Cd$^{2+}$ and Pb$^{2+}$ onto sugarcane bagasse using 2$^3$ factorial designs was studied by [19]. Three operating factors were analysed: temperature (30-50°C), initial metal concentration (0.1 and 1.0 mol dm$^{-3}$) and pH (5 and 6). The fixed parameters were time of exposition (8 h) and initial biosorbent concentration (0.2 g L$^{-1}$). The authors concluded that temperature is the most important factor in the single system (Pb$^{2+}$), while initial metal concentration was the most important variable for the binary system (Cd$^{2+}$ and Pb$^{2+}$). In the single system the adsorption increases with increasing temperature and in the binary one the adsorption decreasing with increasing initial metal concentration. In contrary, the results of the present study showed that temperature was not the most important variable though it acquired significant influence on the adsorption of Pb$^{2+}$. The adsorption of Pb$^{2+}$ increases with decreasing temperature. In addition, interaction effect of T.pH has significant influence on Pb$^{2+}$ removal efficiency.

**CONCLUSIONS**

The factorial experiment design method is undoubtedly good technique for studying the influence of major process parameters on response factors by significantly reducing the number of experiments and henceforth, saving time, energy and money. The use of factorial design offers good and fast screening procedure and mathematically computes the significance of several factors in one experiment that predicts where the optimum is likely to be located. Besides, it allows the identification of the most important parameters for biosorption of metallic ions under tested conditions. In the present research, the most significant effect for Cd$^{2+}$ and Pb$^{2+}$ biosorption was ascribed to pH. The interaction effects of X.pH and T.pH also have a significant influence on the Cd$^{2+}$ and Pb$^{2+}$ removal efficiency, respectively.

The normal probability plot between the predicted values (model) and the observed (experimental) clearly demonstrate how closely the set of observed values with the predicted ones, with high correlation coefficients. In addition, the biosorption studies of Cd$^{2+}$ and Pb$^{2+}$ onto E. crassipes biomass showed that this biosorbent was a powerful and low-cost biosorbent for these metallic ions removal from aqueous solution opening the possibility of this biosorbent to be employed in the treatment of industrial effluents and agricultural waste waters before being delivered into the environment. It is worthwhile to advise the metal industry sponsors to apply such experimental designs to maintain high efficiency and profit biosorption process.

**REFERENCES**


