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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 2^3. The three factors considered were pH, temperature and metal ion concentration at two markedly different levels: 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 X.pH and T.pH 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 disfunction, 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 with 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

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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^{3+} and Cr^{6+} using 2^3 and 2^4 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 hand-held meter (Crison 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:

$$R = \frac{C - C_F}{C}.100 \qquad (1)$$

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

	Element			
	Cd^{2+}		Pb^{2+}	
*Factor	Low level	High level	Low level	High level
T (°C)	20.0	45.0	20.0	45.0
X (mg l^{-1})	10.0	1200.0	10.0	1200.0
pH	2.0	6.0	2.0	6.0

* T: Temperature; X: Initial concentration

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

			Element					
			Cd^{2+}			Pb^{2+}		
Factor			Removal efficiency	(%)*	Average	Removal efficiency	(%)*	Average
T	X	pH						
1	1	1	37.6	37.5	37.6	56.8	50.8	53.8
1	1	-1	19.5	26.2	22.9	28.5	27.4	28.0
1	-1	1	50.0	70.0	60.0	10.0	20.0	15.0
1	-1	-1	10.0	20.0	15.0	70.0	80.0	75.0
-1	1	1	34.8	37.7	36.3	50.3	42.8	46.5
-1	1	-1	24.5	29.5	27.0	88.0	80.0	84.0
-1	-1	1	90.0	80.0	85.0	40.0	20.0	30.0
-1	-1	-1	20.0	30.0	25.0	19.1	23.8	21.5

* Experiment in duplicate

depend on the 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 2^3 factorial design and results for removal efficiency are shown in Table 2. For treatment of data, the Minitab Statistical Software (release 14.1) was employed throughout in order 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, time of contact between the metallic ion and the biosorbent, speed of shaking, etc. The optimization of all those variables using the univariate procedure is very tedious and the best condition could not be attained, because the interactions among all the factors are neglected. Also, it is not known if the set of other fixed variables were kept at other levels, the results would lead to the same optimization. In addition, the total number of experiments to be carried out in the univariate procedure is much higher when compared with statistical design of experiments.

In this study, the factors screened were pH, initial metallic ion concentration (X) and temperature (T), for removal efficiency of Cd^{2+} and Pb^{2+} by *E. crassipes* biomass using a batch adsorption system. Main interaction effect, coefficients of the model, standard error of each coefficient and the probability for the full 2^3 factorial designs for Cd^{2+} and Pb^{2+} are presented in Tables 3 &5, respectively.

The codified mathematical model employed for the 2^3 factorial design is:

$$\begin{aligned} R = A_0 + A_1T + A_2X + A_3pH + A_4TX \\ + A_5TpH + A_6XpH + A_7TXpH \end{aligned} \tag{2}$$

Where A_0 represents the global mean and A_i the other regression coefficients.

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

$$\begin{aligned} RCd^{2+} = 41.36 - 8.64T - 2.94X + 14.94pH \\ + 2.06TX - 10.06TpH + 0.40XpH + 0.39TXpH \end{aligned} \tag{3}$$

$$\begin{aligned} RPb^{2+} = 146.52 - 0.07T - 1.25X - 21.03pH \\ + 0.01TX + 0.02TpH + 0.24XpH + 0.01TXpH \end{aligned} \tag{4}$$

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^{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^{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.

Table 3: Statistical parameters for 2^3 design (Cd^{2+})

Term	Effect	Coefficient	S.E. of coefficient	p
Constant		41.36	0.388	0.006
Main factors				
T	-17.28	-8.64	0.388	0.083
X	-5.88	-2.94	0.388	0.029
pH	29.87	14.94	0.388	0.017
Interaction of two factors				
T.X	4.13	2.06	0.388	0.118
T.pH	-20.12	-10.06	0.388	0.500
X.pH	-0.77	-0.40	0.388	0.025
Interaction of three factors				
T.X.pH	0.78	0.39	0.388	0.550

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

As can be seen from Tables (3&5), some main factors and their interactions were significant at 5% of probability level (p <0.05). On the other hand, some effects were discarded, because they did no exhibit any statistical significance. As such, the resultant models can be represented by:

$$RCd^{2+} = 41.36 - 2.94X + 14.94pH - 0.40XpH \tag{5}$$

$$RPb^{2+} = 146.52 - 0.07T - 21.03pH + 0.02TpH \tag{6}$$

In order to better evaluate each factor and its interaction in case of Cd^{2+}, Fig.1A, presented the normal probability plot of standardized effects. The graph of Cd^{2+} could be divided in two regions: the region with percent below 50%, where the factors and their interactions presented negative coefficients (T, X, X.pH) and the region with percent above 50%, where the factors presented positive coefficients (pH, T.X, T.pH, T.X.pH,). All these factors and interactions which were represented as a square were significant figures while the effects represented by a circle were not significant (Fig.1A).

Fig. 1B presented the Pareto Chart of standardized effects at p = 0.05. All the standardized effects were in absolute values (to verify which were positives and negatives, see Fig. 1A). All the values that presented an absolute value higher than 15.88 (p = 0.05), which were located at right of the line, were significant. The absolute standardized value of the effect of each factor and its interaction appeared at the right of each bar.

Table 4: Analysis of variance for removal efficiency of Cd^{2+} - full 2^3 factorial design (coded units)

Source	d.f.	Seq SS	Adj SS	Adj MS	F	p
Main Effects	3	2450.91	2450.91	816.97	680.10	0.028
2-Way Interactions	3	845.26	845.26	281.76	234.55	0.048
Residual Error	1	1.20	1.20	1.20		
Total	7	3297.38				

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

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. crassipes* biomass was favored at high pH values (pH 6.0). In order to avoid a disruption of the *E. crassipes* 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 statistical design of experiments over the conventional univariate process of optimization of the system. This information would not be acquired in a univariate of optimization in biosorption system. The negative value of X.pH coefficient meant that low metallic 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. crassipes* 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

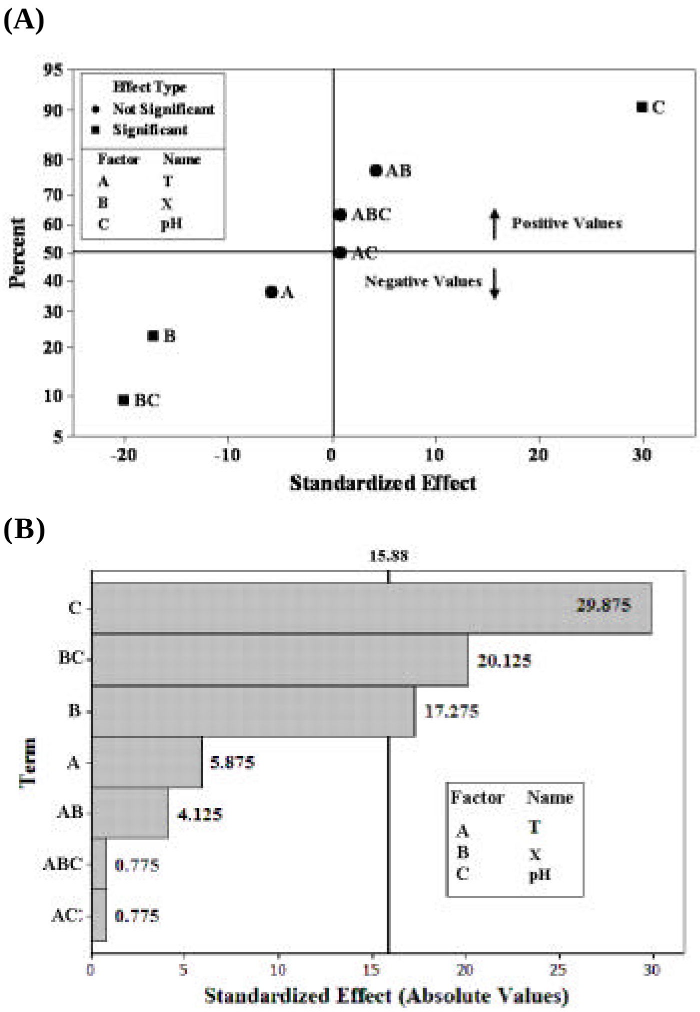

Fig. 1: (A) Cd^{2+} Normal probability plot of standardized effect at p=0.05. The line at 50% divides the negative effects from the positive ones.
(B) Pareto plot of standardized effect (absolute value) at p=0.05

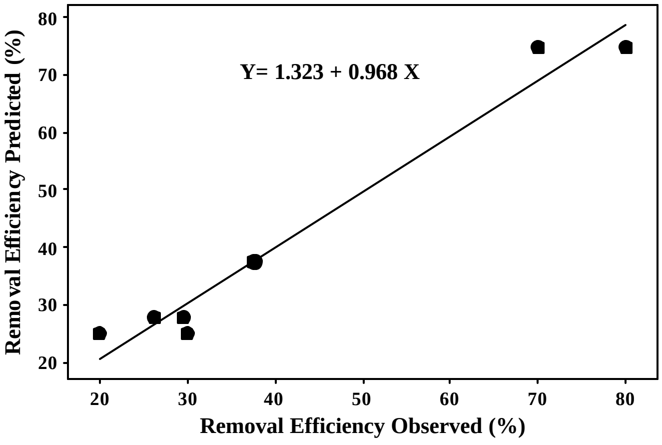

Fig. 2: Normal probability plot for the removal efficiency of Cd^{2+}

Table 5: Statistical parameters for 2^3 design (Pb^{2+})

Term	Effect	Coefficient	S.E. of coefficient	p
Constant		146.52	4.50620	0.020
Main factors				
T	-0.15	-0.07	0.004	0.025
X	-2.50	-1.25	0.124	0.063
pH	-42.06	-21.03	0.936	0.021
Interaction of two factors				
T.X	0.03	0.01	0.001	0.869
T.pH	0.03	0.02	0.001	0.031
X.pH	0.48	0.24	0.025	0.067
Interaction of three factors				
T.X.pH	0.02	0.01	0.001	0.932

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^{2+} - full 23 factorial design (coded units)

Source	d.f.	Seq SS	Adj SS	Adj MS	F	p
Main Effects	3	757.19	2962.82	987.61	309.81	0.042
2-Way Interactions	3	3159.66	3159.66	1053.22	330.39	0.040
Residual Error	1	3.19	3.19	3.19		
Total	7	3920.04				

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

[15, 24-26]. The second important factor for overall optimization of Pb^{2+} biosorption was the temperature (T). Similarly, the negative value of its coefficient justifies that low temperature led to highest biosorption of Pb^{2+}. In Fig. 2 B, though the interaction of two factors T.pH was significant, it acquired the least effect on the removal efficiency of Pb^{2+} 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 2^3, 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 $Cd^{2+}$and Pb^{2+}, 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 Cd^{2+} and Pb^{2+}, respectively.

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 Cd^{2+} was attended at the higher pH value (pH=6) while that for Pb^{2+} 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 Cu^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+}, while that for Pb^{2+} was from the acidic range (pH 2-4). The adsorption of Pb^{2+} at lower pH was also observed in other biomaterials such as the biomass *Zoogloea ramiiger* [30] and fungus *Mucor rouxii* [31]. On the other hand, [15] studied the adsorption capacity of $Pb^{2+,}$ affected by experimental parameters such as pH, contact time and concentration of Pb^{2+} solution, on to *Eichhornia speciosa* plant biomass. They found that the uptake percent of Pb^{2+} increased by increasing pH values. Moreover, [24] reported that Pb^{2+} removal by different organs of *Hemidsmus 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 Cd^{2+}ions from aqueous solution using the green alga (*Ulva lactuca*) biomass were investigated as a function of pH, biomass dosage, contact time and temperature [26]. They found that the maximum biosorption of Cd^{2+}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

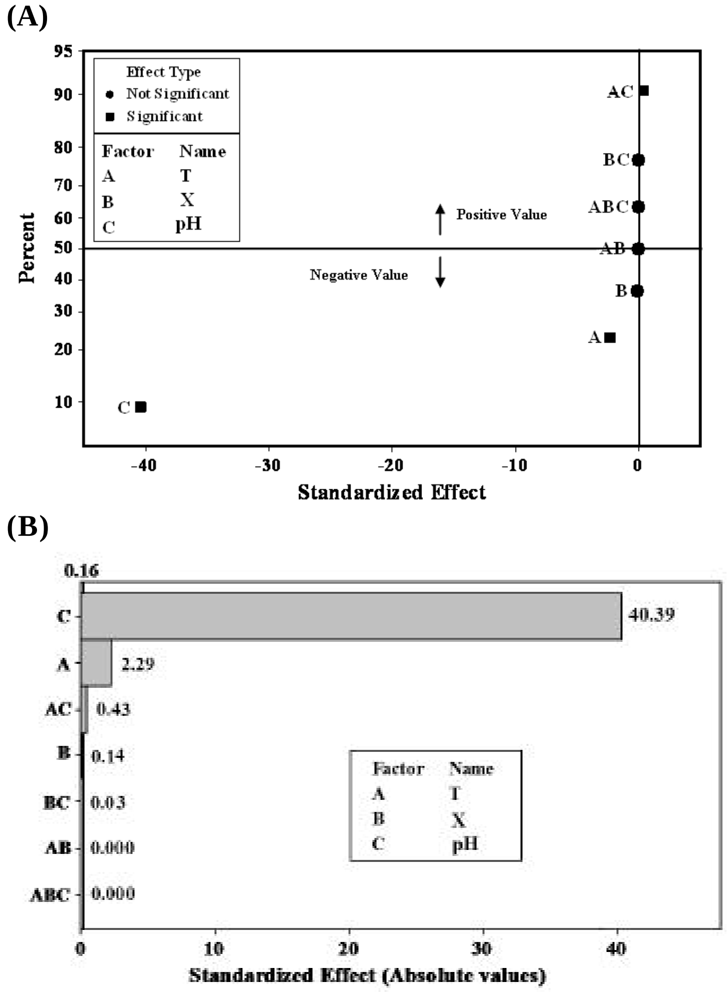

Fig. 3: (A) Pb^{2+} Normal probability plot of standardized effect at p=0.05. The line at 50% divides the negative effects from the positive ones.
(B) Pareto plot of standardized effect (absolute value) at p=0.05

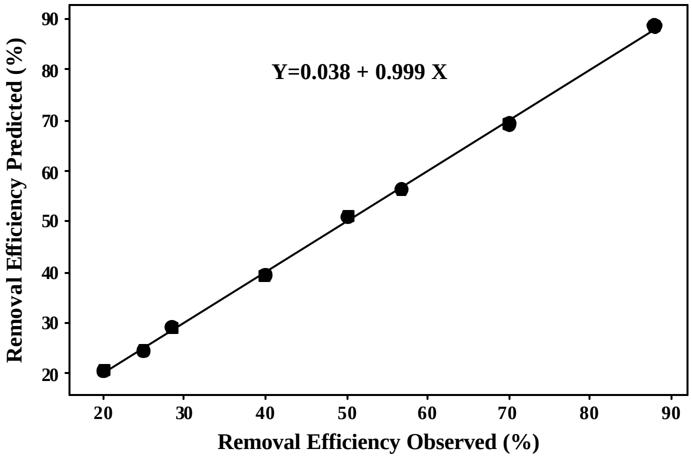

Fig. 4: Normal probability plot for the removal efficiency of Pb^{2+}

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 carboxymethlylated 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.

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