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OPTIMIZATION OF CHROMIUM AND LEAD BIOSORPTION IN WASTEWATER USING 3^3 FACTORIAL DESIGN

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ABSTRACT

In this study removing heavy metals, Cr (III), and Pb (II) from wastewater, Microorganism *Trichoderma* sp. biosorption was performed using Cr (III), and Pb (II) removal was taken into account. For this study, 3^3 Factorial Designs were used, and temperature (°C), biosorbent dosage (g/L), and pH were selected as the main factors for Cr (III), and Pb (II) metals and three levels of these factors were determined as low, medium, and high. In this study, which was carried out to increase the metal removal efficiency and biosorption capacity, the main factors and the significance of each interaction of these factors were examined with 3^3 Factorial Design. For this purpose, by conducting Analysis of Variance (ANOVA) via Response Surface Methodology and optimization, more detailed results were obtained regarding the factors affecting the efficiency of metal removal from wastewater.

Keywords: 3^3 Factorial Design, Optimization, Biosorption, Wastewater, Removal Efficiency

1. INTRODUCTION

Water pollution occurs in the form of a negative change in the physical, chemical, bacteriological, radioactive, and ecological properties of the water supply. Water pollution is a quality change that occurs as a result of anthropogenic effects, restricts or blocks used, and disrupts economic balances. Food and Agriculture Organization of the United Nations (FAO) defines water pollution as the disposal of substances into the water. This proves to be harmful to living resources and dangerous to human health. This pollution prevents activities such as fishing, and it may have detrimental effects on water quality [1].

There is no doubt that the accumulation of heavy metals constitutes the most dangerous dimension of chemical water pollution. The term heavy metal, used synonymously with trace metal, covers trace metals that are essential and those that are not. All of these are potential hazards for living organisms. The most important industrial activities that are effective in the spread of heavy metals to the environment are cement production, iron and steel industry, thermal power plants, glass production, garbage, and waste sludge incineration plants [2].

Heavy metals are toxic when they exceed the concentration limit both in the waters and in the living body where they are found. Especially in the living body, the effect varies depending on the type of creature and the structure of the metal ion, rather than depending on the concentration. For this reason, the maximum concentration restriction has been made, especially in the drinking water of regular consumption and in the food obtained from water sources, and it is necessary to keep it under constant control [3].

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Physical and chemical methods used for heavy metal removal have a limited scope of use due to reasons such as high operating and maintenance costs and additional stress on the environment. Biosorption, in which biological methods are used to remove heavy metals and many other pollutants, is a process that means the removal of pollutants and harmful substances from the environment using microorganisms, such as bacteria, fungi, yeasts, and algae [4,5-6]

In this study, ANOVA via 3^3 Factorial Experiment Design was performed to examine the main and interaction effect of temperature, biosorbent dosage, and pH on the removal efficiency and biosorption capacity from wastewater by removing heavy metals. The main objective was to examine the optimum conditions for the Cr (III) and Pb (II) on two response variables from wastewater. After determining influential factors and their levels, the optimization analyses were applied to evaluate optimum combinations for removal efficiency and biosorption capacity from wastewater.

2. MATERIAL AND METHOD

2.1. Experimental Procedures

The fungal strain *Trichoderma* sp. was isolated from ceramic industrial sludge. One gram of the sludge was inoculated in the potato dextrose broth media amended with heavy metal ions solutions. The strain was isolated on potato dextrose agar media containing (gL^{-1}): agar 15.0, dextrose 20.0, potato extract 4, Streptomycin 0.03, at 25 °C and pH 5.6±0.2. The medium was sterilized by autoclaving at 1.5 atm pressure and 121°C temperature for 20 min. The pure colony was preserved on the slants at 4 °C and identified from morphological characterization with a microscope.

2.2. Experimental Design for Optimization

The aim of this study is to ensure that metal removal efficiency is as high as possible. Therefore, a Full Factorial Design with three levels of three factors was used. Factors affecting Cr (III) and Pb (II) in wastewater were selected as temperature (°C), biosorbent dosage (g/L), and pH, and three levels belonging to these three main factors were determined. These factors and levels are shown in Table 1 for Cr (III) and Pb (II) [7].

Temperature, biosorbent dosage, and pH were selected as the most important factors affecting metal removal efficiency and biosorption capacity as response variables, and a total of 108 experiments were carried out with 4 repetitions for low, medium, and high levels of these parameters.

The three main factors are determined, and three levels of these main factors will be examined with 3^3 Factorial Experiment Design. 27 experimental combinations including temperature (°C), biosorbent dosage (g/L), and pH factors and the interactions of these three factors, coded and quadratic coefficients are given in Table 2. The coded coefficients are 1, 0, and -1, respectively, while the quadratic coefficients are 1, -2, and 1. In addition, since the experiments were carried out in 4 repetitions, a total of 108 response results belonging with observation values were used in the statistical analysis.

Table 1. Factors and Levels for Cr (III) and Pb (II).

Factor	Level 1	Level 2	Level 3
A: Temperature (°C)	20	30	40
B: Biosorbent dosage (g/L)	1	3	5
C: pH	2	4	6

The mathematical model including main factors and their coded and quadratic combinations are given by Equation 1.

$$Y_{ijk} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijk} \quad (1)$$

Where Y represents the response variable (e.g., the removal efficiency or the biosorption capacity), A, B and C are the independent variables (at low, medium and high levels), A_i, B_j, C_k ($i = j = k = 1{,}2{,}3$) represents the estimation of the main effect of the factor, whereas AB_{ij}, AC_{ik} and BC_{jk} represents the estimation of the second order interaction effect between factor i and j, i and k , j and k for the response variable. Moreover, the coefficient μ is constant term, ABC_{ijk} shows the third order interactions' estimation, and finally ε_{ijk} is a random error or residual component [8]. The main hypotheses regarding in this study are given in below.

H_0: A_i=B_j= C_k
H_1: At least one mean value is different from the others.

In the process of creating the experimental design and to obtain the ANOVA results, MINITAB 19 was used. The 3^3 Factorial Design Matrix is given in Table 2.

Table 2. 3^3 Factorial Design Matrix.

Experiment	**Temperature (°C)**		**Biosorbent dosage (g/L)**		**pH**		**Coded Coefficient**			**Quadratic Coefficient**		
	Cr (III)	Pb (II)	Cr (III)	Pb (II)	Cr (III)	Pb (II)	A	B	C	A	B	C
1	20	20	1	1	2	2	1	1	1	1	1	1
2	30	30	1	1	2	2	0	1	1	-2	1	1
3	40	40	1	1	2	2	-1	1	1	1	1	1
4	20	20	3	3	2	2	1	0	1	1	-2	1
5	30	30	3	3	2	2	0	0	1	-2	-2	1
6	40	40	3	3	2	2	-1	0	1	1	-2	1
7	20	20	5	5	2	2	1	-1	1	1	1	1
8	30	30	5	5	2	2	0	-1	1	-2	1	1
9	40	40	5	5	2	2	-1	-1	1	1	1	1
10	20	20	1	1	4	4	1	1	0	1	1	-2
11	30	30	1	1	4	4	0	1	0	-2	1	-2
12	40	40	1	1	4	4	-1	1	0	1	1	-2
13	20	20	3	3	4	4	1	0	0	1	-2	-2
14	30	30	3	3	4	4	0	0	0	-2	-2	-2
15	40	40	5	5	4	4	-1	0	0	1	-2	-2
16	20	20	5	5	4	4	1	-1	0	1	1	-2
17	30	30	5	5	4	4	0	-1	0	-2	1	-2
18	40	40	3	3	4	4	-1	-1	0	1	1	-2
19	20	20	1	1	6	6	1	1	-1	1	1	1
20	30	30	1	1	6	6	0	1	-1	-2	1	1
21	40	40	1	1	6	6	-1	1	-1	1	1	1
22	20	20	3	3	6	6	1	0	-1	1	-2	1
23	30	30	3	3	6	6	0	0	-1	-2	-2	1
24	40	40	5	5	6	6	-1	0	-1	1	-2	1
25	20	20	5	5	6	6	1	-1	-1	1	1	1
26	30	30	5	5	6	6	0	-1	-1	-2	1	1
27	40	40	3	3	6	6	-1	-1	-1	1	1	1

3. RESULTS AND DISCUSSION

3.1. Statistical Analyses

3.1.1. Analysis of Variance and Optimization for Removal Efficiency

In this study three main factors for each of the three levels are determined to apply Full Factorial Design on metal removal efficiency and biosorption capacity from wastewater. Analysis of Variance (ANOVA) was applied to statistically test the effect of these parameters on Cr (III) and Pb (II) biosorption. The full-factorial experimental design matrix in three factors and their levels of the response result were also represented in Appendix section. Moreover, the results of the analyses are shown in Table 3-6.

ANOVA is significant when the higher magnitude of the F-values, and the smaller value of p ($p < 0.0001$). The models F-values of 918.51 and 776.54 indicates that the models are significant Cr (III) and Pb (II) biosorption, showing that the models were perfectly fit with data. P values were obtained from ANOVA for all independent variables shown in Table 3 and 5, in that many variables measured low value ($p < 0.0001$), it revealed the model is significant. The competency of model was checked by analyzing Adj. R-squared (determination coefficient). The model was acceptable to predict the exact correlation between the response and significant variables, it shown the high Adj. R-squared values for two analyses (99.55% and 99.47%) value represented that the variation could be followed in the total response [9].

According to the ANOVA results in Table 3, for removal efficiency of the Cr (III) as a response variable, it was concluded that, main factors of A (temperature), B (biosorbent dosage), C (pH) and their second and third order interactions (A*B*C) have significance effect along with p-values less than 0.05 and High F-values.

Similarly, based to ANOVA results in Table 5 large F-values along with p-values less than 0.05 of main factors and interactions shows that the models are significant on removal efficiency of the Pb (II) from wastewater. In other words, main factors of A, B and C, their interactions (A*B, A*C and B*C) and third order interactions (A*B*C) have critical effect on response variable. Moreover, main effects and interactions in the model explain the removal efficiency of the Pb (II) at a rate of 99.47% as seen in Table 6.

Table 3. ANOVA Table for Removal Efficiency of the Cr (III)

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	26	151169	5814.2	918.51	0.000
Linear	6	135766	22627.7	3574.67	0.000
A	2	86	43.1	6.80	0.002
B	2	7025	3512.7	554.93	0.000
C	2	128655	64327.4	10162.28	0.000
2nd-Order Interactions	12	12653	1054.4	166.57	0.000
A*B	4	2442	610.4	96.43	0.000
A*C	4	2754	688.4	108.76	0.000
B*C	4	7458	1864.4	294.54	0.000
3rd-Order Interactions	8	2750	343.7	54.30	0.000
A*B*C	8	2750	343.7	54.30	0.000
Error	81	513	6.3		
Total	107	151682			

Table 4. Model Summary for Cr (III) on the Removal Efficiency

S	R-sq	R-sq(adj)	R-sq(pred)
2.51595	99.66%	99.55%	99.40%

Table 5. ANOVA Table for Removal Efficiency of the Pb (II)

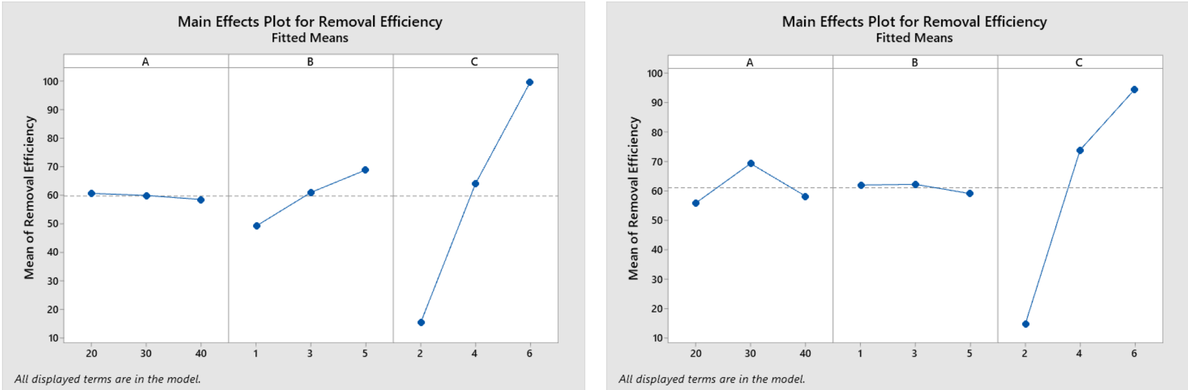
Source	DF	Adj SS	Adj MS	F-value	P-value
Model	26	152086	5849.4	776.54	0.000
Linear	6	127145	21190.8	2813.19	0.000
A	2	3725	1862.5	247.26	0.000
B	2	222	111.0	14.74	0.000
C	2	123198	61598.9	8177.57	0.000
2^{nd}-Order Interactions	12	17625	1468.7	194.98	0.000
A*B	4	2266	566.5	75.20	0.000
A*C	4	1960	490.1	65.06	0.000
B*C	4	13399	3349.7	444.68	0.000
3^{rd}-Order Interactions	8	7316	914.5	121.40	0.000
A*B*C	8	7316	914.5	121.40	0.000
Error	81	610	7.5		
Total	107	152696			

Table 6. Model Summary for Pb (II) on the Removal Efficiency

S	R-sq	R-sq(adj)	R-sq(pred)
2.74457	99.60%	99.47%	99.29%

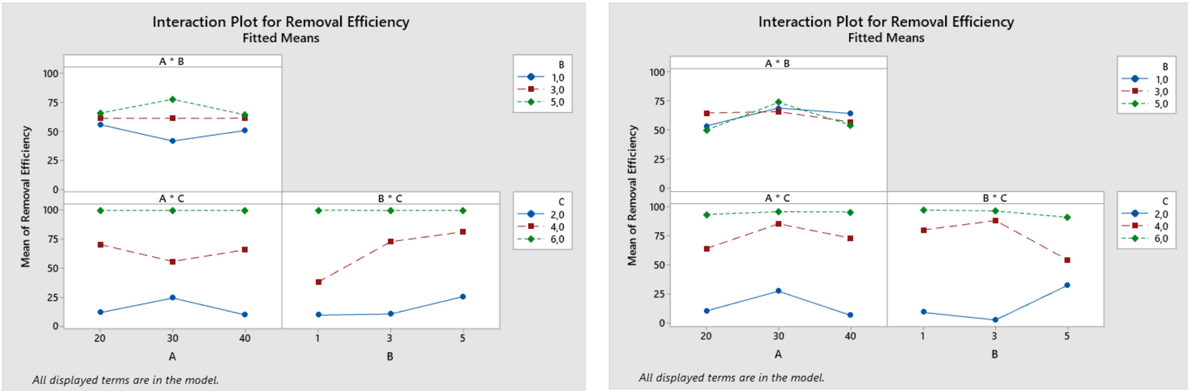
In this part of the study, the main effects of low, medium and high levels of temperature (A), biosorbent dosage (B) and pH (C) for Cr (III) and Pb (II) on removal efficiency are shown in Figure 1(a) and Figure 1(b). In Figure 1(a), it was deduced that all the main factors A, B and C effective on removal efficiency. It can be deduced that the pH (C) has an important influence than biosorbent dosage (B) and temperature (A). Moreover, these results show that, biosorbent dosage (B) and pH (C) positively effects on the removal efficiency, while temperature (A) has negative effect. The results in Figure 1(b) shows the pH (C) has greatest and positively effect on the response variable and on the other hand temperature (A) and biosorbent dosage (B) have the weakest influence on the removal efficiency.

After examining the main effects, the interaction plots were obtained to evaluate every second order interaction effects on removal efficiency for Cr (III) and Pb (II) (Fig. 2(a) and Fig 2(b)) [10]. In these plots, non-parallel lines indicate that the effect of one factor on the response depends on the setting of the other factor. The greater lines depart from being parallel, the greater the strength of the interaction [8,11]. From Figure 2(a), it is seen that there is an interaction between A*B, A*C and B*C as the lines are non-parallel. This means that linear and quadratic combinations of A*B, A*C, and B*C are significant on removal efficiency for Cr (III). In addition to this, Figure 2(b) illustrates the interactions between A*B, A*C and B*C. As the lines non-parallel, it was concluded that there are all interactions are significant effect for Pb (II) on the removal efficiency.



(a) **(b)**

Figure 1. (a): Main effects Plot for Cr (III) on the Removal Efficiency. (b): Main Effects Plot for Pb (II) on the Removal Efficiency.



(a) **(b)**

Figure 2. (a): Interaction Plot for Cr (III) on the Removal Efficiency. (b): Interaction Plot for Pb (II) M on the Removal Efficiency.

According to all the results were obtained, it was concluded that, all the factors considered are important on the Cr (III) and Pb (II) on removal efficiency from wastewater. Accordingly, each main factors and their second and third order interactions should be included in the regression model for optimization. In the next step, the results of the regression and optimization model will be examined with three factors and their interactions for removal efficiency based on the first model.

The optimization analysis was implemented to determine convenient levels of all factors in ANOVA model and to obtain a regression equation for the removal efficiency. The regression equations were obtained in order to maximize Cr (III) (Eq. 2) and Pb (II) (Eq. 3) for removal efficiency response are given in below [12]. Besides, the optimization plots for Cr (III) and Pb (II) on removal efficiency are shown in Figure 3 and Figure 4.

$$y_{Removal\ Efficiency\ for\ Cr} = 59.70 + 1.191A - 9.16B - 39.931C - 3.788AB + 1.251AC - 9.23BC - 3.761ABC \quad (2)$$

Figure 3. Optimization plot for Cr (III) on the removal efficiency.

According to Figure 3, for the optimization of the for Cr (III) on removal efficiency as a response variable, value of the objective function is calculated as 0.99625. This value changes from 0 to 1 and if closer to 1, this means that all factor levels supply optimum conditions [13]. This value (0.99625) also means that determined values belonging to all the factors provide best conditions for removal efficiency from wastewater. In case of A (temperature) 20 °C (low level), B (biosorbent dosage 1 g/L (low level) and C (pH) 6 pH (high level), the maximum value of removal efficiency could reach 99.8870%. As a result, the optimum conditions for Cr (III) on removal efficiency from wastewater are achieved keeping the temperature 20 °C, biosorbent dosage 1 g/L and pH 6 [10].

$$y_{Removal\ Efficiency\ for\ Pb} = 61.115 + 2.905A + 2.023B - 33.446C - 2.25AB + 3.501AC - 1.892BC - 5,.269ABC \quad (3)$$

Figure 4. Optimization plot for Pb (II) on the removal efficiency.

The results of Figure 4 show that, for the optimization of the for Pb (II) on removal efficiency as a response variable, value of the objective function is calculated as 0.98894. This value means that determined values belonging to all the factors provide best conditions for removal efficiency from wastewater. In case of A (temperature) 30 °C (medium level), B (biosorbent dosage) 1 g/L (low level) and C (pH) 6 (high level), the maximum value of removal efficiency could reach 98.0835%. As a result, the optimum conditions for Pb (II) on removal efficiency from wastewater are achieved keeping the temperature 30 °C, biosorbent dosage 1 g/L and pH 6 [10].

3.1.2. Analysis of Variance and Optimization for Biosorption Capacity

Analysis of Variance (ANOVA) was carried out to determine significant main factors and their interactions on biosorption capacity of Cr (III) and Pb (II) from wastewater. ANOVA was also used to identify the statistical significance of the experimental factors on biosorption capacity. The results of ANOVA for biosorption capacity on Cr (III) and Pb (II) are given in Table 7-Table 10.

According to the ANOVA results in Table 7, for biosorption capacity of the Cr (III) as a response variable, it was concluded that, main factors of A (temperature), B (biosorbent dosage) and C (pH) have significance effect ($p<0.05$). Since the F-values are also quite high, factors and interactions are considered statistically significant at %95 confidence level. Additionally, both A*B, A*C and B*C second order interactions, and A*B*C third order interactions have critical effect on biosorption capacity. Besides, when the Adj. R-squared and values were examined, it is seen that main factor and their interactions in the model explain the biosorption capacity of the Cr (III) at the rate of 98.99%.

Similarly, based to ANOVA results in Table 9 large F-values along with p-values less than 0.05 of main factors and interactions shows that the models are significant on biosorption capacity. In other words, based to the ANOVA results in Table 9 main factors of A, B and C, and their interactions (A*B, A*C and B*C) have critical effect on biosorption capacity of the Pb (II) from wastewater. Moreover, main effects and interactions in the model explain the biosorption capacity of the Pb (II) at a rate of 99.88% as seen in Table 10.

Table 7. ANOVA Table for Biosorption Capacity of the Cr (III)

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	26	21271.6	818.14	403.51	0.000
Linear	6	14985.1	2497.51	1231.79	0.000
A	2	67.5	33.76	16.65	0.000
B	2	6151.0	3075.50	1516.85	0.000
C	2	8766.6	4383.29	2161.87	0.000
2nd -Order Interactions	12	5892.4	491.03	242.18	0.000
A*B	4	234.8	58.70	28.95	0.000
A*C	4	252.5	63.12	31.13	0.000
B*C	4	5405.1	1351.28	666.46	0.000
3rd -Order Interactions	8	394.1	49.26	24.30	0.000
A*B*C	8	394.1	49.26	24.30	0.000
Error	81	164.2	2.03		
Total	107	21435.8			

Table 8. Model Summary for Cr (III) on the Biosorption Capacity

S	R-sq	R-sq(adj)	R-sq(pred)
1.42392	99.23%	98.99%	98.64%

Table 9. ANOVA Table for Biosorption Capacity of the Pb (II)

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	26	28198.3	1084.55	2657.06	0.000
Linear	6	22168.2	3694.70	9051.71	0.000
A	2	214.6	107.28	262.83	0.000
B	2	12617.7	6308.85	15456.16	0.000
C	2	9335.9	4667.96	11436.13	0.000
2nd -Order Interactions	12	5714.6	476.22	1166.69	0.000
A*B	4	221.2	55.31	135.50	0.000
A*C	4	133.1	33.28	81.52	0.000
B*C	4	5360.3	1340.07	3283.06	0.000
3rd -Order Interactions	8	315.5	39.44	96.62	0.000
A*B*C	8	315.5	39.44	96.62	0.000
Error	81	33.1	0.41		
Total	107	28231.4			

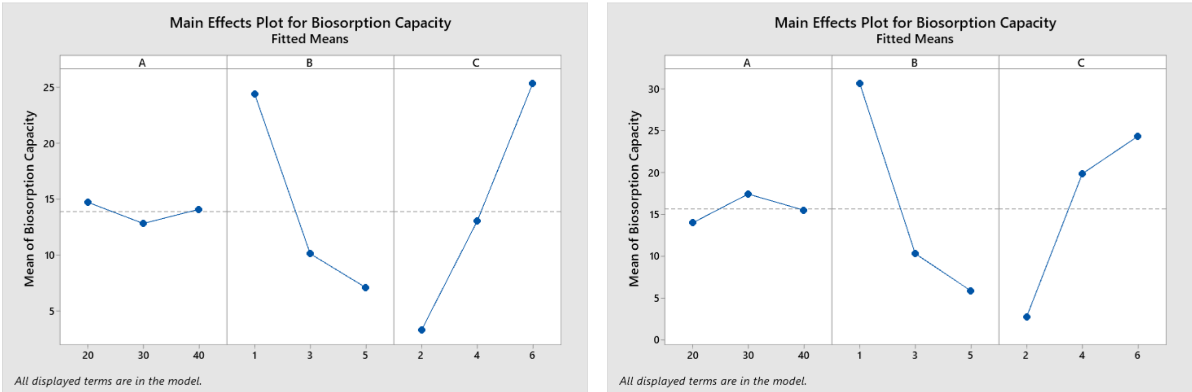
Table 10. Model Summary for Pb (II) on the Biosorption Capacity

S	R-sq	R-sq(adj)	R-sq(pred)
0.638887	99.88%	99.85%	99.79%

In this section, the main effects plots in Figure 5 were obtained shows that A(temperature), B (biosorbent dosage) and C (pH) effect on biosorption capacity for Cr (III) and Pb (II). In Figure 5(a), B and C have most significant effect on biosorption capacity, followed by A. This figure also shows that, B (biosorbent dosage) negatively impacting on biosorption capacity, while C (pH) have positive effect.

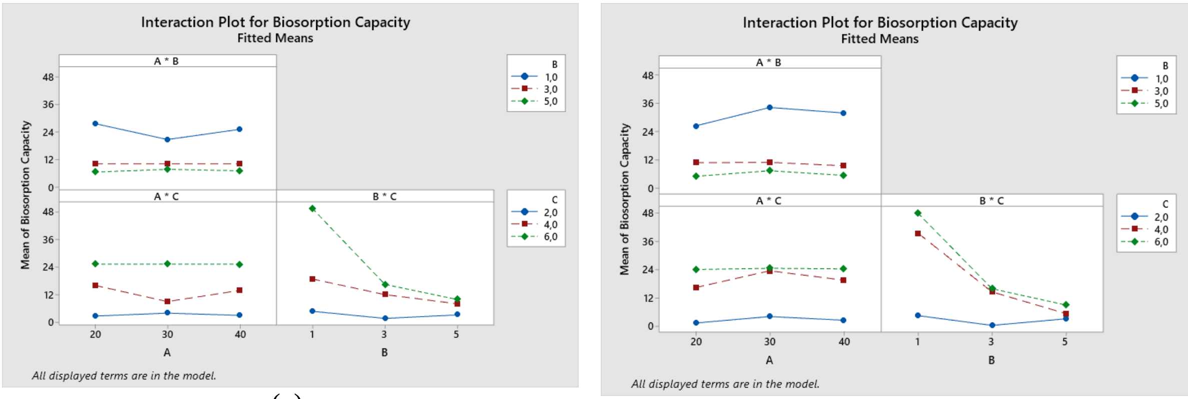
Similarly, In Figure 5(b), it was deduced that all the main factors A, B and C effective on biosorption capacity for Pb (II). Moreover, B (biosorbent dosage) and C (pH) greatest influence on biosorption capacity on the other hand A (Temperature) more less effect than these factors. Additionally, the results in Figure 9. shows both B (biosorbent dosage) and C (pH) have greatest effect on biosorption capacity for Pb (II) and the other hand A (temperature) have weakest effect on response variable. Also, this figure shows that in general, B (biosorbent dosage) has negatively effect on biosorption capacity and on the contrary C (pH) has positive effect.

After analyzing the main effects, the interaction plots for Pb (II) on biosorption capacity were obtained in Figure 6. These figures show that the significant interactions between temperature and biosorbent dosage, temperature and pH and also between biosorbent dosage and pH. In Figure 6(a), it is seen obviously that coded and quadratic combinations of A*B, A*C, and B*C are significant on biosorption capacity for Cr (III) as lines are non-parallel. Similarly, in Figure 6(b), it was concluded that all the interactions (A*B, A*C and B*C) are significant on biosorption capacity for Pb (II).



(**a**) (**b**)

Figure 5. (a): Main effects Plot for Cr (III) on the biosorption capacity. (b): Main Effects Plot for Pb (II) on the biosorption capacity.



(**a**) (**b**)

Figure 6. (a): Interaction Plot for Cr (III) on the biosorption capacity. (b): Interaction Plot for Pb (II) on the biosorption capacity.

According to all the results were obtained, it was concluded that, all the factors considered are important on the Cr (III) and Pb (II) on biosorption capacity from wastewater. Similarly, each main factors and their second and third order interactions should be included in the regression model for optimization. In the next step, the results of the regression and optimization model will be examined with three factors and their interactions for biosorption capacity for Cr (III) and Pb (II).

The optimization analysis was implemented to determine convenient levels of all factors in ANOVA model and to obtain a regression equation for the removal efficiency. The regression equations were obtained in order to maximize Cr (III) (Eq. 4) and Pb (II) (Eq. 5) for removal efficiency response are given in below [12]. Besides, the optimization plots for Cr (III) and Pb (II) on removal efficiency are shown in Figure 7 and Figure 8.

$$y_{Biosorption\ Capacity\ for\ Cr} = 13.896 - 0.21A + 6.779B - 11.44C - 0.223AB - 0.24AC - 8.618BC - 0.256ABC \quad (4)$$

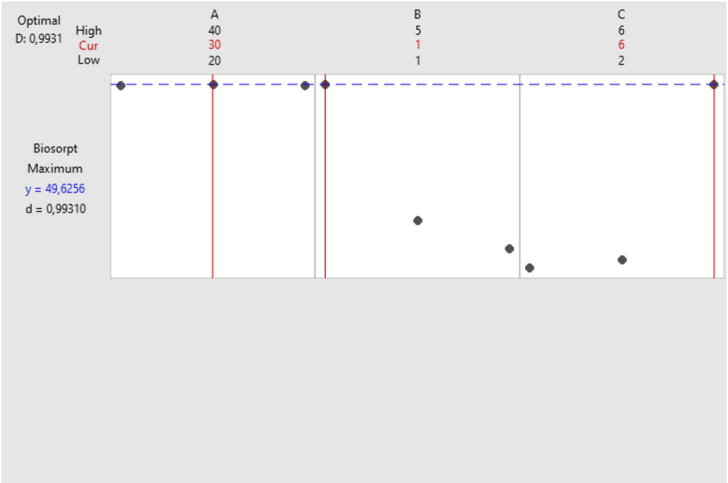


Figure 7. Optimization Plot for Cr (III) on the Biosorption Capacity.

According to Figure 7, for the optimization of the for Cr (III) on biosorption capacity as a response variable, value of the objective function was calculated as 0.99310. This value (0.99310) also means that determined values belonging to all the factors provide best conditions for biosorption capacity from wastewater. In case of A (temperature) 30 °C (medium level), B (biosorbent dosage) 1 g/L (low level) and C (pH) 6 pH (high level), the maximum value of biosorption capacity could reach 49.6256%.

$$y_{Biosorption\ Capacity\ for\ Pb} = 15.6611 + 0.1329A + 9.7593B - 8.6863C - 0.387AB + 0.151AC - 5.541BC - 0.73ABC \quad (5)$$

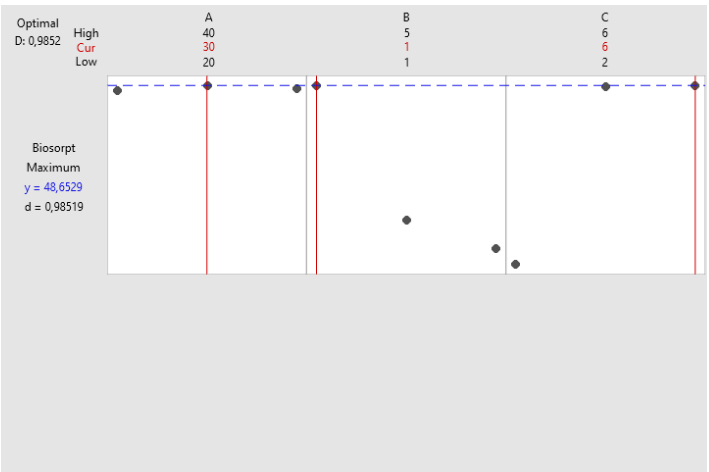


Figure 8. Optimization plot for Pb (III) on the biosorption capacity.

The optimization results of Figure 8 show that, for the optimization of the for Pb (II) on biosorption capacity as a response variable, value of the objective function was calculated as 0.98519. This value means that determined values belonging to all the factors provide best conditions for removal efficiency from wastewater. In case of A (temperature) 30 °C (medium level), B (biosorbent dosage) 1 g/L (low level) and C (pH) 6 pH (high level), the maximum value of biosorption capacity could reach 48.6529%.

4. CONCLUSION

In this study, 3^3 Factorial Experiment Design was applied to investigate the effect of each factor and their interactions) on Cr (III) and Pb (II) on removal efficiency and biosorption capacity. For this purpose, experimental data sets were obtained and the conditions under which the biosorption of Cr (III) and Pb (II) from wastewater yielded the best results were obtained by applying ANOVA and optimization analysis. For this purpose, by conducting ANOVA via Response Surface Methodology and by applying optimization analysis, more detailed results were obtained regarding the conditions under which biosorption of Cr (III) and Pb (II) from wastewater yielded the best results.

The ANOVA results show that temperature (°C), biosorbent dosage (g/L) and pH, and three levels belonging to these three main factors have significance effect for Cr (III) and Pb (II) on removal efficiency and biosorption capacity from wastewater. In addition to this, it was concluded that the most effective factors for the Cr (III) on removal efficiency as a response variable were the biosorbent dosage and pH, while the most influential factors for Pb (II) were temperature and pH. Similarly, while the most effective factors for the biosorption capacity response variable on Cr (III) was biosorbent dosage and pH, the same factors were also effective for the Pb (II). The main effects and their interactions in the ANOVA model also explain the removal efficiency and biosorption capacity response variables with quite high Adj. R-squared values. Moreover, based on interaction plots, all the second and third order interactions have significant effect for Cr (III) and Pb (II) on removal efficiency and biosorption capacity.

Optimization results were obtained depending on ANOVA results, on the other hand, show that all the factors' levels were obtained maximize the removal efficiency and biosorption capacity from wastewater in general. The optimum factors levels are temperature of 20 °C, biosorbent dosage of 1 g/L and pH of 6 for Cr (III) on removal efficiency. However, these levels are temperature of 30 °C, biosorbent dosage of 1 mg/L and pH of 6 for Pb (II) on removal efficiency. On the other hand, the optimum factor levels for Cr (III) on biosorption capacity was detected to be temperature of 30 °C, biosorbent dosage of 1 g/L and pH of 6. Also, in case of temperature of 30 °C, biosorbent dosage of 1 g/L and pH of 6, for the Pb (II) on biosorption capacity reach gave the highest value.

Consequently, in case the factor levels were considered (chosen) as indicated, optimum conditions were provided for both response variables in efficiency of metal removal from wastewater. With this study, it has been demonstrated that Factorial Experimental Design are quite applicable in the field of chemical experiments, and it is predicted that all these experiences make an example for other researchers.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

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APPENDIX

Table 11. 3^3 Factorial Design Matrix for The Removal Efficiency of The Cr (III).

Experiments	Temperature (°C)	Biosorbent dosage (g/L)	pH	Response Values 1. Repetition	2. Repetition	3. Repetition	4. Repetition
1	20	1	2	11.555	9.890	9.822	99.363
2	20	1	4	56.118	56.945	57.782	9.935
3	20	1	6	99.897	99.902	99.868	56.206
4	20	3	2	11.821	11.063	11.001	99.880
5	20	3	4	71.404	70.581	71.851	11.846
6	20	3	6	99.645	99.832	99.570	73.935
7	20	5	2	13.694	14.644	13.146	99.872
8	20	5	4	82.897	82.221	83.377	14.244
9	20	5	6	99.758	99.545	99.485	82.306
10	30	1	2	10.356	10.696	10.551	99.603
11	30	1	4	14.111	14.152	14.545	10.294
12	30	1	6	99.929	99.601	99.926	14.676
13	30	3	2	11.307	10.322	10.588	99.924
14	30	3	4	69.584	76.437	69.584	10.717
15	30	3	6	99.871	99.203	99.045	76.437
16	30	5	2	53.610	51.805	52.373	98.738
17	30	5	4	80.756	80.154	79.893	51.604
18	30	5	6	99.747	100.236	98.819	79.959
19	40	1	2	7.085	6.955	9.583	99.497
20	40	1	4	45.149	48.834	26.627	11.123
21	40	1	6	99.852	99.803	99.983	52.421
22	40	3	2	10.745	10.021	9.554	99.898
23	40	3	4	76.228	71.023	75.180	10.463
24	40	3	6	99.719	99.329	99.848	71.473
25	40	5	2	8.473	11.978	11.059	99.805
26	40	5	4	83.351	80.758	80.410	12.783
27	40	5	6	99.747	99.647	9.822	79.697

Table 12. 3^3 Factorial Design Matrix for The Removal Efficiency of The Pb (II).

Experiments	Temperature (°C)	Biosorbent dosage (g/L)	pH	Response Values 1. Repetition	2. Repetition	3. Repetition	4. Repetition
1	20	1	2	2.000	1.980	2.900	4.200
2	20	1	4	63.540	62.620	56.920	61.040
3	20	1	6	95.964	96.044	96.148	96.280
4	20	3	2	2.000	1.980	2.900	4.200
5	20	3	4	93.710	93.454	92.400	93.396
6	20	3	6	97.548	97.094	97.616	97.792
7	20	5	2	29.840	23.640	20.660	29.640
8	20	5	4	34.900	37.420	37.420	39.780
9	20	5	6	88.972	83.746	84.902	85.190
10	30	1	2	9.800	11.260	12.100	9.360
11	30	1	4	97.360	97.322	97.382	97.586
12	30	1	6	98.349	98.987	97.684	97.314
13	30	3	2	2.000	1.980	2.900	4.200
14	30	3	4	97.636	97.638	97.896	97.528
15	30	3	6	97.042	96.214	95.814	95.722
16	30	5	2	70.500	67.152	71.860	66.020
17	30	5	4	59.900	60.580	53.800	66.240
18	30	5	6	95.930	90.588	90.744	90.634
19	40	1	2	11.920	17.600	15.400	12.840
20	40	1	4	80.160	76.260	83.566	83.958
21	40	1	6	97.460	94.250	99.158	95.628
22	40	3	2	2.000	1.980	2.900	4.200
23	40	3	4	75.230	73.240	71.120	71.120
24	40	3	6	93.270	95.094	95.652	94.334
25	40	5	2	2.000	1.980	2.900	4.200
26	40	5	4	77.720	61.680	60.160	59.560
27	40	5	6	91.742	90.958	96.284	98.064

Table 13. 3^3 Factorial Design Matrix for The Biosorption Capacity of The Cr (III).

				Response Values			
Experiments	**Temperature (°C)**	**Biosorbent dosage (g/L)**	**pH**	**1. Repetition**	**2. Repetition**	**3. Repetition**	**4. Repetition**
1	20	1	2	5.709	4.877	4.882	4.958
2	20	1	4	27.671	28.246	28.548	27.770
3	20	1	6	49.552	49.555	49.245	49.642
4	20	3	2	1.961	1.839	1.833	1.968
5	20	3	4	11.901	11.756	11.919	12.314
6	20	3	6	16.552	16.561	16.507	16.590
7	20	5	2	1.368	1.459	1.310	1.420
8	20	5	4	8.276	8.209	8.318	8.214
9	20	5	6	9.960	9.931	9.941	9.952
10	30	1	2	5.106	5.274	5.254	5.137
11	30	1	4	6.972	7.006	7.258	7.251
12	30	1	6	49.964	49.503	49.371	49.664
13	30	3	2	1.882	1.713	1.7576	1.781
14	30	3	4	11.574	12.680	11.551	12.706
15	30	3	6	16.623	16.512	16.464	16.608
16	30	5	2	5.357	5.170	5.235	5.152
17	30	5	4	8.056	7.996	7.970	7.977
18	30	5	6	9.959	9.964	9.854	9.923
19	40	1	2	3.521	3.464	4.735	5.528
20	40	1	4	22.440	24.271	13.208	26.002
21	40	1	6	49.530	49.310	49.497	49.357
22	40	3	2	1.790	1.665	1.447	1.734
23	40	3	4	12.679	11.813	12.480	11.865
24	40	3	6	16.587	16.522	16.564	16.546
25	40	5	2	0.847	1.196	1.105	9.984
26	40	5	4	8.325	8.060	8.022	7.944
27	40	5	6	9.929	9.965	9.928	9.946

Table 14. 3^3 Factorial Design Matrix for The Biosorption Capacity of The Pb (II).

				Response Values			
Experiments	**Temperature (°C)**	**Biosorbent dosage (g/L)**	**pH**	**1. Repetition**	**2. Repetition**	**3. Repetition**	**4. Repetition**
1	20	1	2	0.996	0.980	1.433	2.079
2	20	1	4	31.33	31.000	28.067	30.278
3	20	1	6	47.134	47.359	47.317	47,948
4	20	3	2	0333	0.329	0.481	0.698
5	20	3	4	15.546	15.514	15.349	15.504
6	20	3	6	16.236	16.129	16.226	16.255
7	20	5	2	2.979	2.360	2.062	2.958
8	20	5	4	3.479	3.904	3.738	3.972
9	20	5	6	8.869	8.368	8.463	8.509
10	30	1	2	4.851	5.585	6.002	4.634
11	30	1	4	48.583	48.179	48.401	48.406
12	30	1	6	48.979	48.810	48.263	48.560
13	30	3	2	0.332	0.329	0.481	0.700
14	30	3	4	16.186	16.208	16.294	16.211
15	30	3	6	16.163	15.961	15.948	15.911
16	30	5	2	7.039	6.696	7.169	6.591
17	30	5	4	5.973	6.041	5.378	6.608
18	30	5	6	9.582	9.041	9.056	9.045
19	40	1	2	5.901	8.765	7.639	6.344
20	40	1	4	39.527	37.903	41.125	41.979
21	40	1	6	48.152	46.751	49.381	47.624
22	40	3	2	0.332	0.330	0.483	0.700
23	40	3	4	12.488	12.205	12.166	11.798
24	40	3	6	15.473	15.838	15.889	15.639
25	40	5	2	0.200	0.197	0.290	0.419
26	40	5	4	7.744	6.158	5.997	5.954
27	40	5	6	9.145	9.085	9.613	9.783