Al-Nahrain University, College of Engineering Journal (NUCEJ) Vol.18 No.2, 2015 pp.328 - 342

Process Optimization of Biosorption Hg(II), Cu(II) and Ni(II) Ions onto Dead Anaerobic Biomass using a Two-Level Full Factorial Design and Response Surface Methodology, Batch Systems

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Abstract:

Dead anaerobic biomass prepared from drying bed wastewater treatment plant were used as adsorbents for the biosorption studies of mercury, copper and nickel ions from synthetic wastewater. The main and interactive effects on uptake of Hg(II), Cu(II) and Ni(II) in this study are investigated through the model equations designed by a two-level full factorial design. Experiments designed by central composite design were carried out and the process response was modeled. Heavy metals removal efficiency and uptake have sequence order Hg(II) > Cu(II) > Ni(II) under tested conditions, Hg(II) offers the strongest component that able to displace Cu(II) and Ni(II) from their sites, while Ni(II) ions was the weakest adsorbed component. Best removal efficiencies were 96.2, 90.3 and 82.4 when temperature 40^0C, pH 6, initial metal ion concentration 10 mg/l, biomass loading 6 g/l, contact time 180 min, and 200 rpm for Hg(II), Cu(II) and Ni(II) respectively.

The results predicted using factorial regression model showed high values of regression coefficients (R^2) 0.945 indicating good agreement with experimental data. The main biosorption mechanisms were complexation and physical adsorption onto natural active functional groups. It is observed that biosorption of these metals was a surface process.

Keywords: Biosorption, Dead Anaerobic Biomass, Heavy Metals, RSM.

1. Introduction

Due to the increase of industrial activities, the contamination by heavy metals in aquatic environments is worldwide problem [1]. Therefore, the contamination by heavy metals, remaining as alarming pollutants due to their nondestructive, toxicity, carcinogenic or mutagenic effects, bioaccumulation and subsequent bio magnification, has received special attention even at very low concentrations [2]. Especially for aquatic environments, with the rapid development of various industries such as metal plating, electroplating, mining, pigment manufacturing, leather, ceramic, batteries, photography, automobile, refrigeration industries, the industrial effluents containing heavy metals drain into the river leading to various diseases in the ecosystem [3, 4].

The heavy metals are among the most common pollutants found in industrial effluents. Solid and/or liquid wastes containing toxic heavy metals may be generated in various industrial processes and they are toxic even at low concentration [5]. World Health Organization (WHO) recommended maximum 0.005, 2, and 0.07 mg/L as acceptable concentrations of mercury, copper, and nickel ions, respectively, in drinking water Due to that, various treatment technologies had been searched to reduce the concentration of heavy metals in the environment [6]. Several heavy metals removal technologies including ion exchange, chemical precipitation, reverse osmosis, ultrafiltration, electrodialysis, and pyhtoremediation are costly technologies with a 10-450 US$ per million liter cost for treated water [1]. However, these technologies are becoming uneconomical and unfavourable to remove heavy metals from industrial wastewaters because they are expensive, large amount of excess sludge produced during the process will cause disposal problem, metal hydroxides formed clogged the membrane, so that a need of cost effective technologies are essential [1,4,**7**]. Recently, focused of using microbial biomass such as bacteria, yeast, molds and algae as a biosorbent for metals ions removal from contaminated effluent [4, 5,7]. Removal and recovery of heavy metals are very important with respect to environmental and economic considerations. Biosorption with low-cost materials (industrial or agricultural residues) has been found to be superior to other techniques in virtue of the low-priced cost, high efficiency, easiness of operation, regeneration of biosorbent and possibility of metal recovery[4,7].

In recent years, biosorption studies have more focused on single metal system than multi-metal system despite the fact that industrial effluents often contain several metal ions concomitantly [1,5] when more than one metal is present, the studies become very complicated since the interaction of one metal biosorption from other metal ions may be synergistic or non-interactive. The traditional one-factor-at-a-time experiments cannot successfully predict possible interactions between the metal ions in aquatic systems. Thus, it is necessary to develop a new method for exploring multi metal sorption [4]. Three experimental design methodologies, factorial design (FD), response surface design (RSM) and mixture design (MD) were used to interpret the characteristics of three metals removal from single and multi-metal aqueous solution by DAB. Factorial design was useful for screening many factors to find the significant few and estimating main effect and interactions [3]. Response Surface Methodology (RSM) has an important application for analyzing effects of several independent variables and also interactive effects among the variables on the response [2,3]. RSM was used to predict the biosorption results under different metal compositions [3,8].

In this study, dead anaerobic biomass (DAB) was first used to remove heavy metals from synthetic wastewater, the biosorption characteristics of Hg(II), Cu(II), and Ni(II) onto DAB in single and multi-metal systems, respectively. The main objectives were (1) to apply a six factor, two-level factorial design and quadratic equations for finding the most significant effect for each parameters including temperature, pH, initial metal ions concentration, biomass loading, contact time and agitation speed, (2) to estimate the affinity of three heavy metal removal and the adsorption capacity onto the DAB as biosorbent in multi-metal system with RSM and mixture designs, (3) to estimate the mechanism of biosorption processes using FT-IR test.

2. Experimental Work

2.1. Materials

2.1.1 Adsorbate

A stock solution of mercury, copper, and nickel ions with a concentration of 1,000 mg/l was prepared by using $HgCl_2$, $Cu(NO_3)_2$ and $Ni(NO_3)_2$ (BDH, England with minimum purity 99.5%). The salts were obtained from local market. About 1.353, 2.951 and 3.113 g of mercury chloride, copper nitrate and nickel nitrate were dissolved in 1,000 ml distilled water, respectively. Metal concentrations were determined by a flame atomic absorption spectrophotometer AA (Type, Buck, Accusys 211, USA) for Cu(II) and Ni(II), while cold vapour atomic absorption spectroscopy (CVAAS) was used for Hg(II). Physicochemical properties of pollutants were summarized in **Table 1.**

2.1.2 Adsorbent

Heterogeneous cultures including mostly anaerobic bacteria, yeast fungi, and protozoa of sorbents were taken from the third extension drying bed in Al-Rostomia'a Treatment Plant/Baghdad-Iraq. The physical, chemical, and biological properties were measured and listed in **Table 2**. Anaerobic and facultative anaerobic micro-organisms (*Aeromonas species, E-coli,Pseudomonas aerginrsa, Clostridium, Staphylococcus sp.and Salmonella sp., and Rhizopusarrhizus, Saccharomyces erevisiae*) were found in biomass from the drying bed using API Instrument (Biomerieux, France). DAB was prepared using heterogeneous culture of live anaerobic biomass (LAB), dried at temperature between 37 and 45°C for five days, crushed, sieved, washed with distilled water, and then dried at 70°C for 6 h equivalent diameter was between 0.4-0.6 mm.

2.2 Batch Biosorption Experiments

Each experiment was carried out in 250 mL Erlenmeyer flask containing 100 mL heavy-metal solution of Hg(II), Cu(II) and Ni(II) whose concentration was set according to experimental design methodology. The initial pH of the solution was adjusted to the required value by adding HNO_3 (0.1 M) and NaOH (0.1M) solutions. The removal rate and amount of adsorbed metal ions (q_e) per gram of DAB were calculated using **Eq.(1 and 2).**

$$\text{removal rate} = \frac{(C_0 - C_e)}{C_0} \times 100 \qquad \text{….. (1)}$$

$$\text{uptake amount (mg/g)} = \frac{(C_i - C_e)V}{M} \qquad \text{….. (2)}$$

2.3 Factorial Design Methodology

Factorial design is usually used for screening important variables and has successfully been applied by researchers [3,4]. Main effects refer to the effect of a single factor on a specific experimental response, averaged across the levels of any other independent factors. In contrast, factor interactions occur when the effect of a factor depends on the level setting of another factor. All factorial designs are able to identify main effects as well as factor interactions all experimental variables are used in sifting main effects, leading to the most efficient use of resources and the effects are evaluated by a wide range of conditions with the minimum of resources. [9].

In order to evaluate the factors that affected the removal rate (%) of heavy metals (mercury, copper, and nickel ions), a minimum run resolution v characterization design was applied. Six factors, pH, initial metal ions concentration, biomass loading, temperature, contact time and agitation speed were studied.

2.4 Response Surface Methodology (RSM)

In recent years, RSM has been generally applied to optimize experimental conditions. However, the RSM consists of a group of empirical techniques devoted to the evaluation of relationship existing between a cluster of controlled experimental factors and measured responses according to one or more selected criteria [3,4,9].

RSM explores the relationships between several explanatory variables and one or more response variables. The optimal response was found using a second-degree polynomial model. This model is only an approximation, but use it because such a model is easy to estimate and apply, even when little is known about the process [10] the true functional relationship between the dependent variable (response) (Y) and the set of independent variables (factors) (X_1, X_2, . .). If knowledge concerning the shape of the true response surface is insufficient, first attempts generally try to approximate the shape by fitting a first-order model to the response values. However, if the first-order model suffers from lack of fit arising from existence of surface curvature, the first-order model is upgraded by adding higher order terms to it. The next higher order model is the second-order model and is given by **Eq. (3)**:

$$\mathrm{Y} = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{k}\sum_{j=1}^{k} \beta_{ij} X_i X_j + \varepsilon \quad \ldots\ldots (3)$$

where Y is the predicted response, X_i, X_j, X_k are the input variables, which affect the response Y, X_1^2 , X_2^2 ,…, X_k^2 are the square effects, β_0 is the intercept term, X_iX_j, X_jX_k, and X_iX_k, are the interaction effects, β_i (i = 1, 2, k) is the linear effect, β_{ii} (i = 1, 2, k) is the squared effect, β_{ij} (j = 1, 2, k) is the interaction effect and ε is a random error [2,11]

3. Results and Discussion

3.1 Screening of the Parameters by Minimum Run Resolution V Characterization Design

The factors and their respective level are summarized in **Table 3**. Factors were chosen to study the response as removal rate (%) of heavy metals by biosorption onto DAB. Each factor was represented at two levels high and low, denoted by (+1) and (−1) signs, respectively [3,4]. **Table 4** shows the removal efficiency for each metal ion.

The design matrix of uncoded values for the tested factors and the observed responses in terms of the removal rate (%) of Hg(II), Cu(II), and Ni(II) for all experimental runs including replicates, are shown in **Table 4**. The analysis of variance (ANOVA) for biosorption study of Hg(II), Cu(II), and Ni(II) onto DAB was used in order to ensure a good model. The test for significance of regression model and the results of ANOVA are summarized in **Table 5**. Prob> F less than 0.05 indicated model terms were significant [3,4]. The predicted R^2 and adjusted R^2 values were 0.863 and 0.838, 0.831 and 0.786, 0.889 and 0.862 in reasonable agreement for Hg(II), Cu(II), and Ni(II), respectively. Best removal efficiencies were 96.2, 90.3 and 82.4 when temperature 40^0C, pH 6, finial metal ion concentration 10 mg/l, biomass loading 6 g/l, contact time 180 min, and agitation speed 200 rpm for Hg(II), Cu(II) and Ni(II) respectively. The adequate precision value was the “signal to noise ratio”. A ratio greater than 4 was desirable [4,9]. A ratio of 22.9 for Hg(II), 17.6 for Cu(II) and 13.8 for Ni(II) obtained indicated an adequate signal. Therefore, these models can be used. The regression models obtained minimum run resolution v characterization design represented the effect of the tested factors on the responses. The removal efficiencies models proposed are as follows as actual factors in **Eqs. (4 to 6)**

Hg(II) removal efficiency (%) =
-27.229+7.310(pH)+5.441(BM)+0.156 (CT)+0.103(AS) ….. (4)

Cu(II) removal efficiency (%) =
-12.107+3.885(pH)+2.644(BM)0.043(CT)+0.0577(AS) +0.038(pH)(CT) ….. (5)

Ni(II) removal efficiency (%) =14.282+1.816(pH)–0.0981(CT)+0.0534(pH)(CT) ….. (6)

Where:
pH= pH value of the solution
BM= biomass loading, g/l
CT= Contact time, min
AS= Agitation speed, rpm

As it is clear from **Eqs. (4 to 6)** and **Figure 1**, that pH, biomass loading, contact time and agitation speed have a main effect on the response for Hg(II) and Cu(II) removal, while biomass and contact time have a main effect on response for Ni(II) removal this is due to the properties of metal ions as it is illustrated in **Table 1**. In view of above mentioned conclusions, medium temperature for 30°C, pH for 4, biomass loading for 3.25 g/L, contact time for 95, and agitation speed for 110 rpm were used for exploring the behavior of multi metal biosorption in following RSM and mixture designs.

3.2 Data Analysis from Response Surface Methodology (RSM) Design

Results obtained from multi metal biosorption experiments are listed in **Table 6**. The face-centered composite design (FCCD) which was a modified center composite design was chosen to explore the behavior of multi-metal biosorption onto DAB [4]. The responses are uptake capacities for Hg(II), Cu(II) and Ni(II) onto DAB using **Eq. (2)**. The design contained a total of 20 experiments, the experimental design of response surface obtained by Box-Behnken [12]. Models were evaluated by the analysis of variance (ANOVA), the uptake capacities models proposed are as follows as actual factors in **Eqs. (7 to 9).** The F-value with allow probability value ($P < 0.0001$) demonstrates a high significance for three regression models. The determination coefficient obtained at 95% of confidence level was 0.985 for Hg(II), 0.931 for Cu(II) and 0.904 for Ni(II), revealing that these regressions were statistically significant

$$\mathbf{Hg(II)}\text{uptake (mg/g)} = 4.67 + 0.89\,X_1 - 0.46\,X_2 - 0.31\,X_3 - 0.012\,X_1X_2 - 6.89 \times 10^{-3}X_1X_3 + 4.32 \times 10^{-3}X_2X_3 + 3.82 \times 10^{-3}X_1^2 + 8.1 \times 10^{-3}X_2^2 + 4.54 \times 10^{-3}X_3^2 \quad \ldots\ldots (7)$$

$$\mathbf{Cu(II)}\text{uptake(mg/g)} = 4.06 - 0.21\,X_1 - 0.55\,X_2 - 0.29\,X_3 - 5.96 \times 10^{-3}X_1.X_2 + 2.01 \times 10^{-3}X_1.X_3 - 2.88 \times 10^{-3}X_2.X_3 + 3.6 \times 10^{-3}X_1^2 + 1.36 \times 10^{-3}X_2^2 + 3.9 \times 10^{-3}X_3^2 \quad \ldots\ldots (8)$$

$$\mathbf{Ni(II)}\text{uptake(mg/g)} = 2.78 - 0.41\,X_1 - 0.15\,X_2 + 0.57\,X_3 - 2.36 \times 10^{-3}X_1.X_2 - 3.72 \times 10^{-3}X_1.X_3 - 4.24 \times 10^{-3}X_2.X_3 + 8.41 \times 10^{-3}X_1^2 + 5.1 \times 10^{-3}X_2^2 - 1.63 \times 10^{-3}X_3^2 \quad \ldots\ldots (9)$$

Where:
X_1= Hg(II) concentration, mg/l
X_2= Cu(II) concentration, mg/l
X_3= Ni(II) concentration, mg/l

The response surface contour plots for ions uptake were created using the design-expert 9 software for predicting binary and ternary metal biosorption onto DAB. The predicted profiles for the selective biosorption of Hg(II), Cu(II) and Ni(II) are illustrated in **Figure 2** respectively. Each contour plot represented a number of combinations of two test variables holding the other variable at middle levels [13]. **Figure 2(a)** shows the simultaneous effect of initial Hg(II) and Cu(II) concentration on the Hg(II) uptake in binary system. The Hg(II) uptake decreased slightly with increase of initial Cu(II) concentration. Hg(II) uptake on DAB was more than 30 mg/g when Hg(II) and Cu(II) concentration were 37-50, 0-19 mg/l respectively, while Hg(II) uptake was more than 30 mg/g in Hg(II)-Ni(II) system when Hg(II) and Ni(II) concentration were 43-50, 0-16 mg/l respectively.

The uptakes of Cu(II) onto DAB in Cu(II)-Hg(II) and Cu(II)-Ni(II) binary systems were more than 20 mg/g when Hg(II) and Ni(II) increases from 0-18 mg/l and 0-14 mg/l respectively. The uptakes of Ni(II) in Ni(II)-Hg(II) and Ni(II)-Cu(II) binary systems were more than 15 mg/g when Hg(II) and Cu(II) increases from 0-11 mg/l and 0-7 mg/l respectively, as shown in **Figure 2 (b, c)**.

Figure 3 demonstrate the uptake of each ions in ternary system, that is to say, the present of Ni(II) and Cu(II) had a little suppression of Hg(II) uptake in multi-metal system. In addition, Cu(II) concentration showed a remarkable effect while a little effect of Ni(II) concentration was observed. These results indicated that in all cases, there was an inhibitory effect of one metal on binding of other metals, with Hg(II) showing the greatest effect. However, the uptake order in ternary system was found to be Hg(II) > Cu(II) > Ni(II). The findings which were in agreement with previous studies [14,15] it can be explained by the physicochemical properties of metal ions (**Table 1**). Hg(II) has higher atomic weight and lower solubility in water than Cu(II) and Ni(II), while Cu(II) and Ni(II) have the same coordination number, charge, similar diamagnetism and similar electronegativity, which imply that the two ions would compete similarly with the Hg(II). Moreover, Hg(II) has a greater affinity to biomass, which may be due to the fact that the metal has the largest atomic weight, the most electronegativity, Generally, the metals with the highest uptake capacity in the mono-component system showed greater inhibitory effect on the biosorption of other metal ions in the multiple-component system [1,5].

3.3 Data Analysis from Mixture Designs

The experiments were carried out with the same conditions as RSM. The result of ANOVA showed that the larger F-values (8234.51 for Hg(II), 345.57 for Cu(II) and 1004.33 for Ni(II)) and the smaller P-values (<0.001) indicated the more significant model term. The square of correlation coefficient for each response was computed as the R-square (R^2) that is a measure of the amount of variation around the mean explained by the model. The values of R^2 (0.924 for mercury, 0.941 for copper and 0.975 for nickel) and correspondingly adjusted R^2 (0.826 for mercury, 0.867 for copper and 0.942

for nickel) that indicated a high correlation between the observed values and the predicted values.

Figure 4 describes the uptake of Hg(II), Cu(II) and Ni(II) onto DAB for ternary system with a total metal concentration of 150 mg/L. The triangular contour plots show that the preference of DAB on biosorption of a target metal from multi- metal system was in the order of Hg(II) > Cu(II), Hg(II) > Ni(II) and Cu(II) > Ni(II). Therefore, the affinity of DAB for three metals followed as: Hg(II) > Cu(II) > Ni(II).

The design points mainly matched the predicted surfaces, suggesting that the augmented simplex-lattice design could predict accurately the behavior of multi-metal biosorption. These indicate that the interaction of the target metal with its co-existing metal ions in multi-metal biosorption was greatly similar no matter what the total metal concentration same results were obtained by Yan-ru, and Malihe [3,4]. Besides, when the total metal concentration was known in wastewater, the individual adsorption capacity of three metals can be predicted from the 3D surface plots.

In this study, according to the results of RSM and two mixture designs, maximum metal uptake capacities onto DAB were found as 57.3, 33.2 and 27.6 mg/g for mercury, copper and nickel, respectively.

3.4 Effect of Functional Groups

In order to understand the surface binding mechanism, it is essential to identify the functional groups present on the biomass involved in this process. The main effective binding sites can be identified by FT-IR spectral. The main functional groups proposed for the metal uptake are amino, carbonyl, carboxylic, hydroxyl, phosphate and others, mainly those from polyssacharidic material which constitutes most of the cell wall [16,17]. The spectra were measured within the range of 400–4000 cm^{-1}. However, FT-IR spectroscopic analysis showed strong bands at 3150–2900 cm^{-1}, which is indicated of (–OH) as the hydroxyl groups. The peaks around 1650 cm^{-1} show the carbonyl (-C=O) stretching vibration of the carboxyl groups of amino acids, peaks ranging from 1300 to 1000 cm^{-1} are described generally to the (C–O) stretching vibration in carbonayl and alcohols [16]. The results show that Hg (II), Cu(III) and Ni(II) may be adsorbed or complexed by H and O atoms of hydroxyle and carboxylic bonds, which shifted the bands to lower frequencies. These shifts may be attributed to the changes in counter ions associated with hydroxyle, carboxylic of amino acids and, carboxylate, these results agreed with [18]. **Figure 7** shows FT-IR spectra results the order of biosorption of heavy metals removed by complexation mechanisms on the surface of biomass is the following: Hg(II) > Cu(III) > Ni (II).

4. Conclusions

Biosorption of Hg(II), Cu(II) and Ni(II) on to DAB was investigated in single, binary and ternary systems. The following conclusions can be drawn from this study: (1) Medium pH, biomass loading, contact time and agitation speed were found to have a main effect on the removal of the three metals by screening the six independent variables with the run resolution v characterization design.

(2) According to Face-Centered Composite (FCCD) design and augmented simplex-lattice design, the biosorption preference onto DAB was following the order of Hg(II)>Cu(II)>Ni(II) for binary and ternary system. Mercury ions could still be effectively removed from aqueous solution in the presence of both copper and nickel ions while removal of the copper and nickel ions would be suppressed by mercury.

5. References

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Table 1: Main physicochemical properties of the metals tested

Properties	**Mercury**	**Copper**	**Nickel**
Formula	Hg(II) from HgCl2	Cu(II) from Cu(NO$_3$)$_2$	Ni(II) from Ni(NO$_3$)$_2$
Appearance	colorless or white solid	blue crystals	emerald green crystals
Molar mass, g/mole	271.52	187.56	182.70
Standard atomic weight	200.59	63.55	58.69
Solubility in water, g/100 mL	7.4	137.8	94.2
Hydrated radius (Å)	4.19	4.19	4.04
Ionic radius (Å)	1.02	0.69	0.72
Electronegativity	1.9	1.9	1.8
Charge	2	2	2
Density, g/cm^3	5.43	3.05	2.05
Wavelengths used by AA, nm	334	324.7	249.2
Company	BDH (England)	BDH (England)	BDH (England)

Table 2: Physical chemical and biological characteristic of DAB

Physical characteristic (dead biomass)		Biological characteristic (live biomass)	
Particle diameter, mm	0.775	*Bacteria*	
Surface area, m^2/g	94.53$^{(a)}$	*Aeromonas species, CFU/mL*	222000
Actual density, kg/m	1741.6	*E-coli, CFU/mL*	430000
Bulk density, kg/m	609.9$^{(b)}$	*Pseudomonas aerginrsa, CFU/mL*	703500
Particle porosity	0.584	*Klebsiella species, CFU/mL*	210000
Total Suspended Solid, mg/L	153950	*Clostridium, CFU/mL*	370000
Volatile Suspended, mg/L	78126	*Staphylococcus sp., CFU/mL*	210000
Chemical characteristic (dead biomass)		*Streptococcus sp., CFU/mL*	490000
pH	5.5-6.3	*Salmonella sp., CFU/mL*	190000
CEC, meq/100g	51.2	*Shiglla dysente, CFU/mL*	410000
Mercury, mg/L	0.02	*Fungi*	
Copper, mg/L	0.03	*Penicillium sp., CFU/mL*	180000
Nickle, mg/L	0.01	*Yeast*	
		Candida albicans, CFU/mL	460000
		Protozoa	
		Entamoeba species, CFU/mL	16000
		Giardia lambihia, CFU/mL	90000

a Surface area analyzer, BET method, Quantachrome.com.(USA), (b) Apparent density instrument, Autotap, Quantachrome.(USA), CEC Cat ion Exchange Capacity, CFU Colony-forming unit

Table 3: Factors and levels used in the minimum run resolution vcharacterization design

Factor	**Name**	**Units**	**Level and range (coded)**	
			-1	1
A	Temperature	°C	20	40
B	pH	--------	2	6
C	Initial metal ions concentration	mg/L	10	50
D	Biomass loading	mg/L	0.5	6
E	Contact time	Min	10	180
F	Agitation speed	rpm	20	200

Table 4: Minimum run resolution v characterization design for determination of the most important variables affecting the metal ions removal

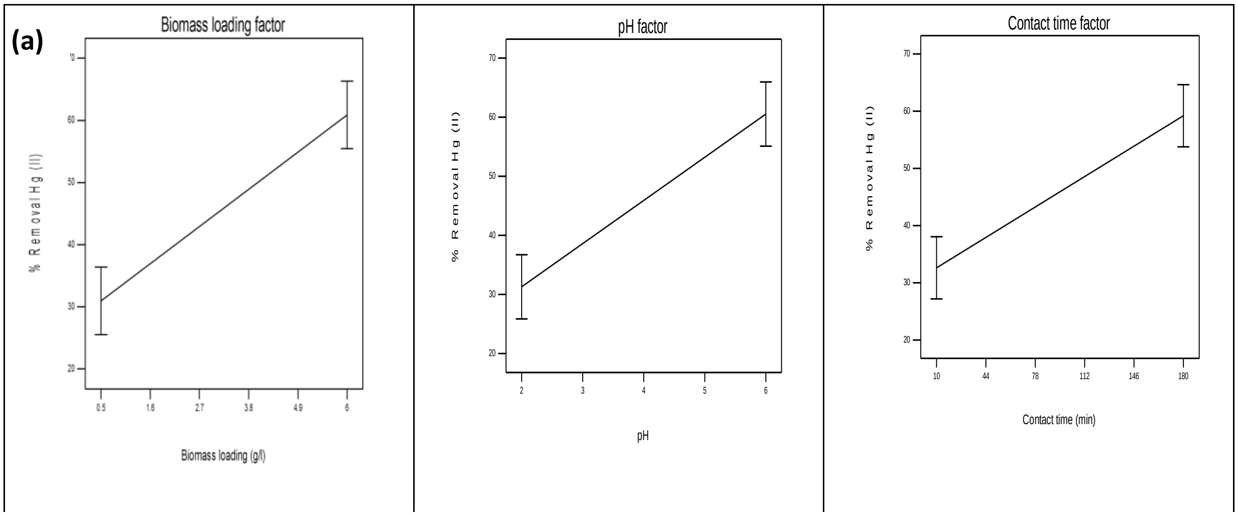
runs	**Temperature (°C)**	**pH**	**Initial metal ions (mg/L)**	**Biomass loading (mg/L)**	**Contact time (min)**	**Agitation speed (rpm)**	**Responses (% removal)**		
							Hg(II)	**Cu(II)**	**Ni(II)**
1	20	2	50	6	10	200	37.7	28.3	26.2
2	40	6	10	0.5	10	20	26.8	13.9	25.5
3	20	2	10	0.5	180	20	28	9	13
4	40	6	50	6	10	20	50.8	29	25.5
5	20	6	10	6	10	200	80.9	29	25.5
6	40	6	50	0.5	10	200	40.8	24.4	24.6
7	40	2	10	6	10	200	50.1	17.1	15.8
8	20	6	10	6	180	20	73.9	66.4	66.6
9	40	2	50	0.5	10	20	9.7	7.4	18.7
10	40	2	50	6	180	200	67	31	23.2
11	40	2	10	6	180	20	75.5	9.6	21.2
12	40	6	10	6	180	200	96.2	90.2	82.4
13	20	6	10	0.5	180	200	90.2	66.7	66.7
14	40	6	50	0.5	10	20	20.4	19	35.5
15	20	2	50	6	180	20	40.4	27.1	27.3
16	40	2	10	0.5	180	200	33	6.8	10.3
17	20	6	50	0.5	10	20	15.2	27.7	27.8
18	20	2	10	0.5	10	200	6.6	6.2	12.7
19	20	2	10	6	10	20	10.2	8.1	16.6
20	20	6	50	6	180	200	94.9	65.7	64.4
21	40	6	50	0.5	180	20	38.2	20.4	45.8
22	20	2	50	0.5	180	200	23.6	15.3	21.8

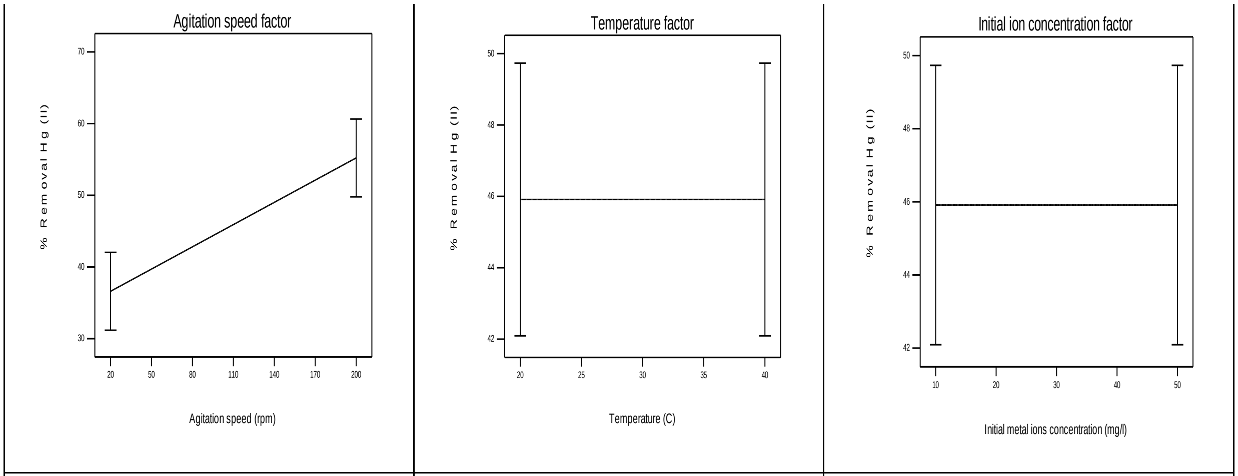
Table 5: Analysis of variance F value and P_{rob}>F for removal efficiencies of three metals

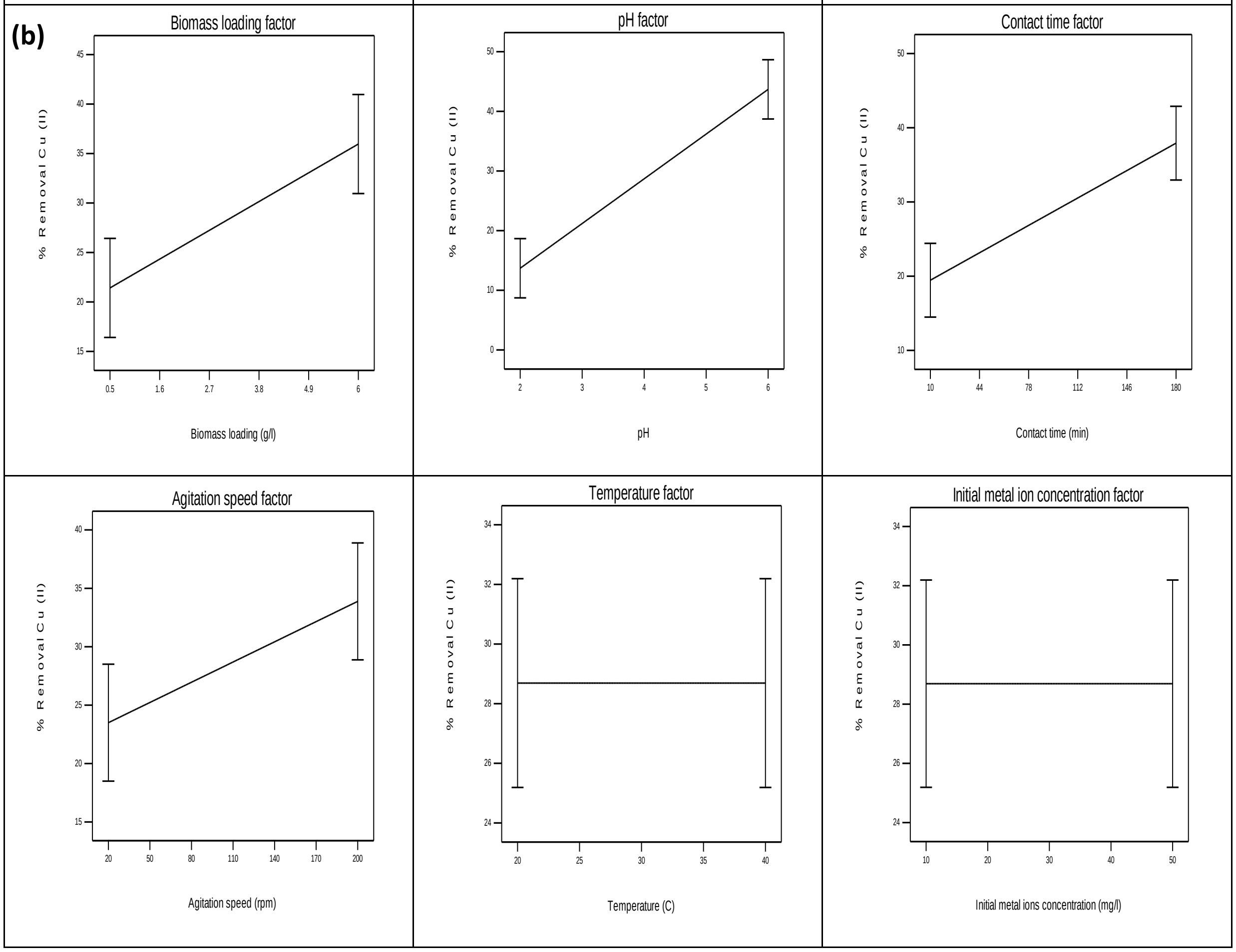
	Hg(II) removal efficiency		Cu(II) removal efficiency		Ni(II) removal efficiency	
	F Value	p-value $P_{rob} > F$	F Value	p-value $P_{rob} > F$	F Value	p-value $P_{rob} > F$
Source Model	26.11	< 0.0001	15.88	< 0.0001	42.20	< 0.0001
B-pH	31.90	< 0.0001	40.67	< 0.0001	69.44	< 0.0001
D-Biomass loading	33.41	< 0.0001	9.29	0.0077	35.21	< 0.0001
E-Contact time	26.37	< 0.0001	15.43	0.0012	30.15	< 0.0001
F-Agitation speed	12.91	0.0022	4.73	0.0449	42.20	< 0.0001
B-pH-E-Contact time			7.18	0.0164	69.44	< 0.0001

Table 6: list of experimental design of response surface using box-Behnken and uptake results according to surface methodology for single and multi-metal biosorption onto DAB

runs	Hg(II) mg/l	Cu(II) mg/l	Ni(II) mg/l	Hg(II) uptake mg/g	Cu(II) uptake mg/g	Ni(II) uptake mg/g
1	0	0	25	0	0	16.1
2	50	0	25	48.1	0	9.4
3	0	50	25	0	27.3	18.7
4	50	50	25	17.3	12.4	6.1
5	0	25	0	0	19.2	0
6	50	25	0	36.8	11.4	0
7	0	25	50	0	9.2	21.3
8	50	25	50	19.56	6.44	12
9	25	0	0	27.5	0	0
10	25	50	0	12.4	23.9	0
11	25	0	50	15.8	0	17.9
12	25	50	50	11.5	16.7	7.3
13	25	25	25	8.77	7.38	3.66
14	25	25	25	8.72	6.77	3.84
15	25	25	25	8.92	7.92	5.14
16	25	25	25	8.72	6.72	4.1
17	25	25	25	8.92	6.3	4.62
18	25	25	25	9	6.46	3.23
19	25	25	25	8.87	6.61	3.66
20	25	25	25	8.96	6.35	4.5




Agitation speed factor
% Removal Hg (II)
70
60
50
40
30
20
50
80
110
140
170
200
Agitation speed (rpm)
Temperature factor
% Removal Hg (II)
50
48
46
44
42
20
25
30
35
40
Temperature (C)
Initial ion concentration factor
% Removal Hg (II)
50
48
46
44
42
10
20
30
40
50
Initial metal ions concentration (mg/l)


(b)
Biomass loading factor
% Removal Cu (II)
45
40
35
30
25
20
15
0.5
1.6
2.7
3.8
4.9
6
Biomass loading (g/l)
pH factor
% Removal Cu (II)
50
40
30
20
10
0
2
3
4
5
6
pH
Contact time factor
% Removal Cu (II)
50
40
30
20
10
10
44
78
112
146
180
Contact time (min)
Agitation speed factor
% Removal Cu (II)
40
35
30
25
20
15
20
50
80
110
140
170
200
Agitation speed (rpm)
Temperature factor
% Removal Cu (II)
34
32
30
28
26
24
20
25
30
35
40
Temperature (C)
Initial metal ion concentration factor
% Removal Cu (II)
34
32
30
28
26
24
10
20
30
40
50
Initial metal ions concentration (mg/l)

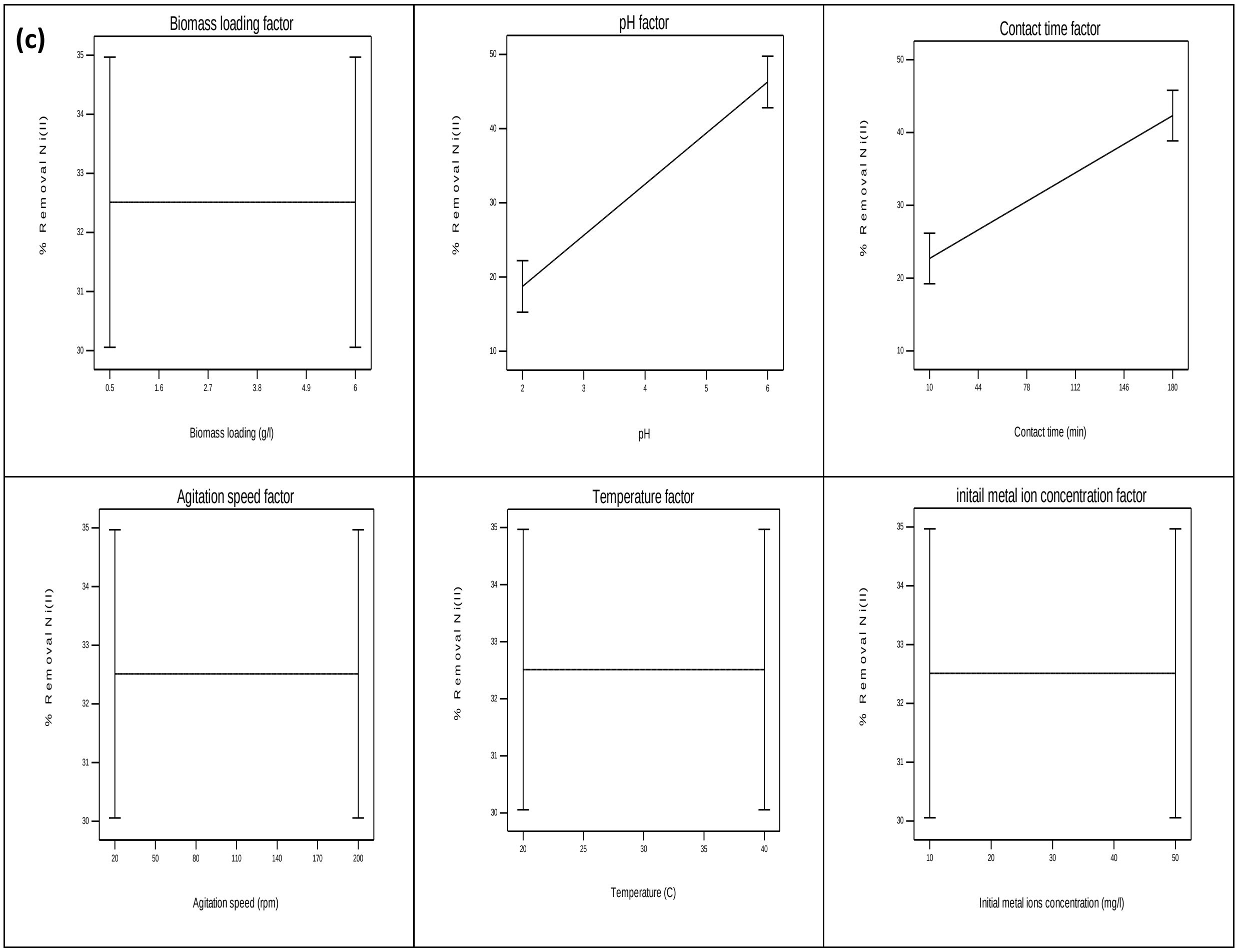


Figure 1: Main effects models for removal of a) Hg(II), b) Cu(II), c) Ni(II) onto DAB, temperature 30^{o}C, pH 4, initial metal ion concentration 30 mg/l, biomass loading 3.25 g/l, contact time 95 min and agitation speed 110 rpm

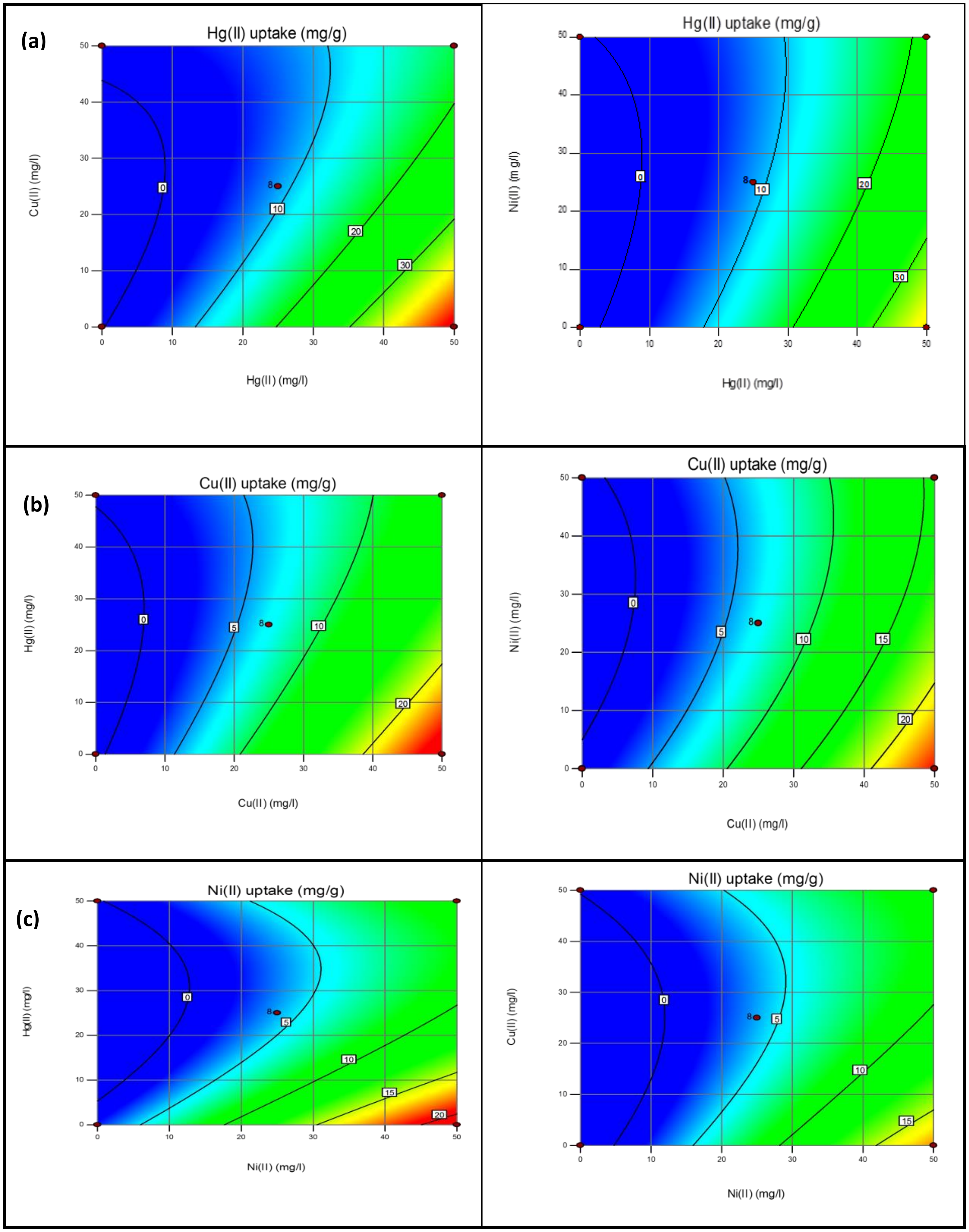


Figure 2: Response surface contour plot for competitive biosorption of a) Hg(II) b)Cu(II) and c)Ni(II) with other two metals ions onto DAB in binary system holding another concentrations at central values 25 mg/l, temperature 30°C, pH 4, biomass loading 3.25 g/l, contact time 95 min and agitation speed 110 rp

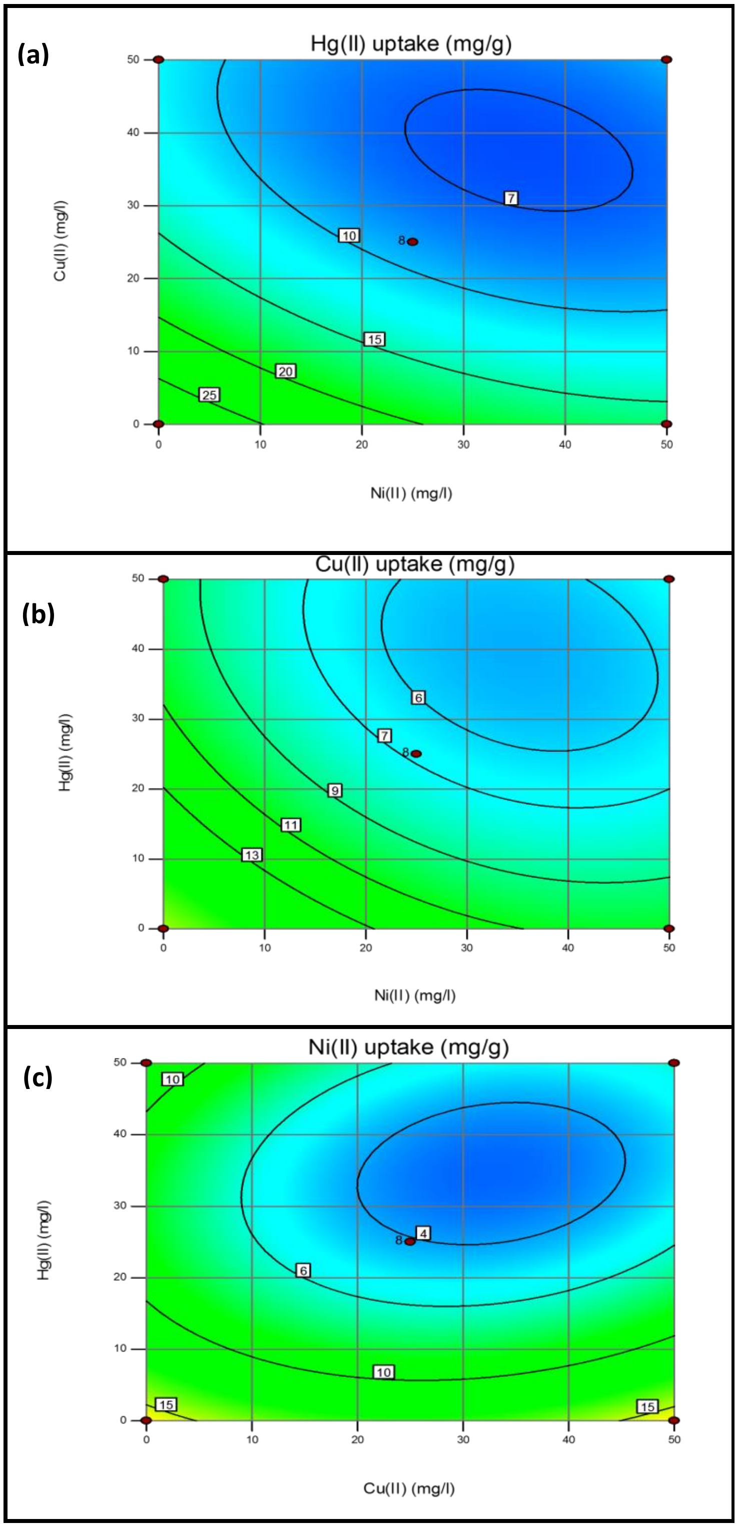


Figure 3: response surface contour plot for competitive biosorption of a)Hg(II) b)Cu(II) and c)Ni(II) onto DAB in binary system holding another concentrations at central values 25 mg/l, temperature 30°C, pH 4, biomass loading 3.25 g/l, contact time 95 min and agitation speed 110 rpm

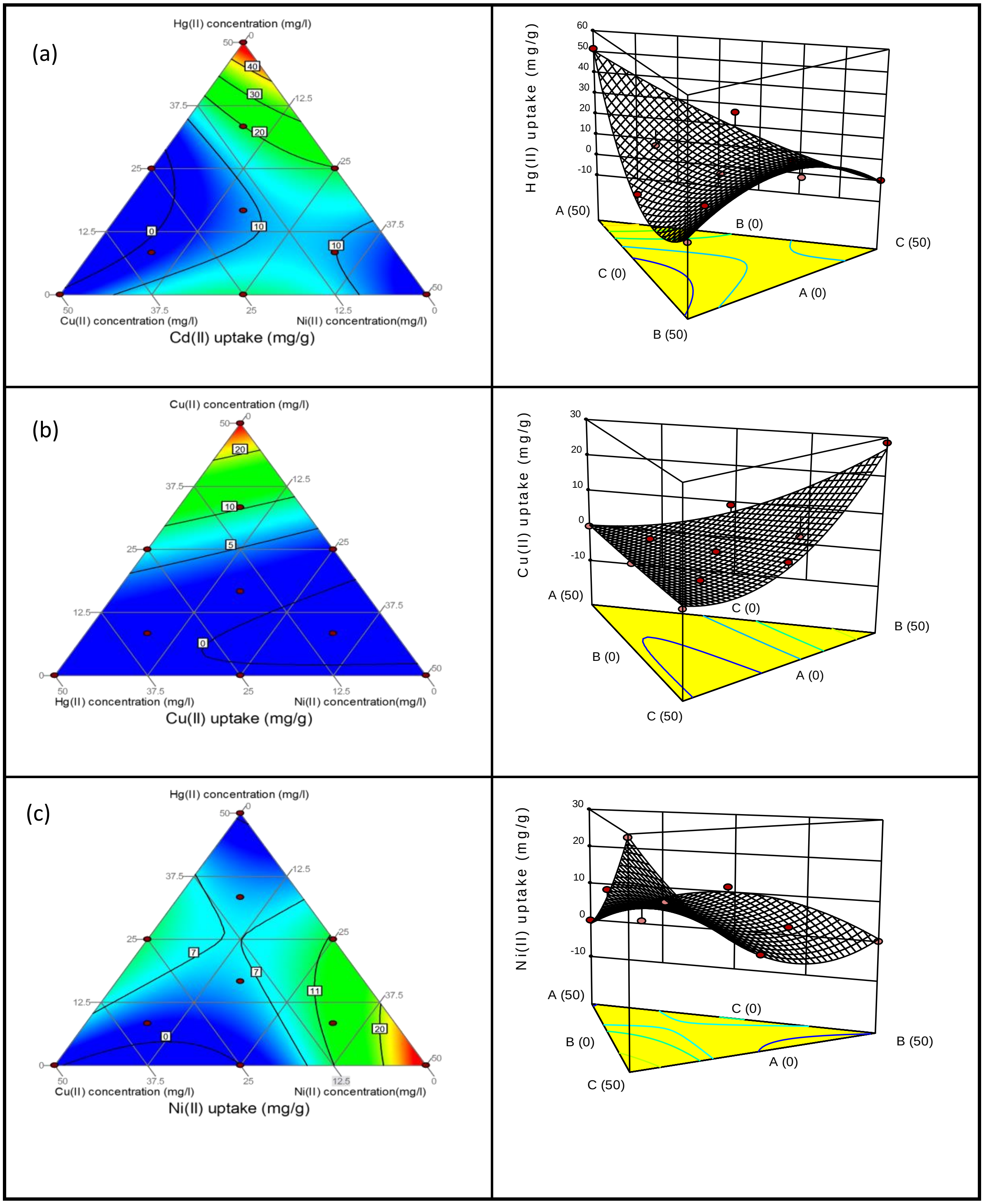


Figure 4: Graphical representation for the biosorption of Hg(II), Cu(II) and Ni(II) on to DAB in ternary system, triangular contour diagrams and triangular three-dimensional biosorption surfaces, the values indicated in (a) , (b) and (c) were biosorption capacities in a unit of mg/g).

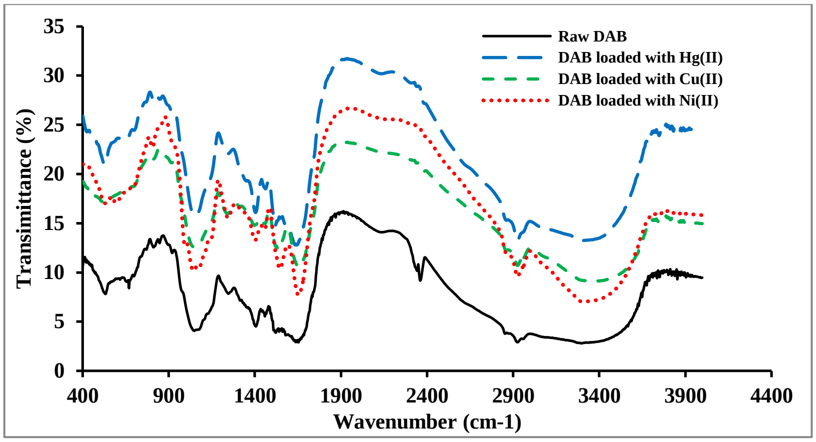


Figure 5: FT-IR spectra for raw DAB biomass before and after loaded with 50 mg/L of Hg(II), Cu(II), and Ni(II) ions; pH 5, Dp 0.4-0.6 mm single system

عملية تحسين الامتزازا الحيوي لايونات الزئبق ،النحاس والنيكل باستخدام الكتلة اللاهوائية الميتة باستخدام اثنين من مستوى كامل المضروب وتصميم ومنهجية الاستجابة السطحية، نظام الدفعة

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الخلاصة:-

استخدمت الكتلة الحيوية اللاهوائية الميتة التي أعدت من احواض تجفيف محطة معالجة مياه الصرف الصحي كمادة ممتزة. تم دراسة قابيلة هذه المادة لامتزاز ايوانات كل من الزئبق, النحاس والنيكل من المياه الصناعية, وتم دراسة العوامل الرئيسة الموثرة على عملية الامتزاز وذلك من خلال دراسة العوامل الرئيسة الداخلة في هذه العملية تم تحقيق هذه الدراسة من خلال نموذج المعادلات باستخدام (two-level factorial design, central composite) design بعد ذلك تم مقارنة النتائج العملية مع النتائج االمتنبا بها . وجد ان إزالة المعادن الثقيلة وكفاءة امتصاص اخذت التسلسل الزئبق >النحاس>النيكل, في ظل ظروف اجراء التجارب وجد ان ايونات الزئبق ذات أقوى قابلية على المتزاز باستخدام الكتلة اللاهوائية الميتة وايضا وجد ان له القدرة على إزاحة ايونات كل من النحاس والنيكل من مواقعها وتحل محلها بصورة تنافسية، في حين وجد ان أيونات النيكل أضعف عنصر قابل على الامتزاز .كانت أفضل كفائة للإزالة هي 96.2، 90.3 و 82.4 عند درجة حرارة $40^{0}C$، ودرجة الحامضية 6، تركيز أيون المعدن 10 ملغ / لتر و الكتلة الحيوية 6غرام / لتر، وقت 180 دقيقة، و 200 دورة في الدقيقة .النتائج النهائية وباستخدام نموذج الانحدار أظهرت توافق عالي يصل الى 0.945 مما يدل على تطابق جيد مع البيانات التجريبية .وكانت آليات الرئيسية للامتزاز الايونات هي functional groups لوحظ أن الامتزاز الحيوي لهذه الايونات هي بصورة رئيسية تحدث على السطح.