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Optimization of Ni (II) removal on Rhizomucor *tauricus* by using Box-Behnken design

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Abstract

Rhizomucor tauricus is used in the production of lipase, after several cycles it is disposed off as biological waste. A new biosorbent material fungus, *Rhizomucor tauricus* was immobilized, used as an adsorbent for removal of Nickel (II) and for optimization Box-Behnken design used by statistica 6.0. The time required to reach equilibrium was found to be 4 hours. The optimized concentration is 60 mg/L, pH is 6, biomass volume was 20 with high biosorption capacity of 449 mg/g. FTIR spectral analysis indicated that the amide – NH bending, CH stretching, carboxylic acid, and hydroxylic groups are the main functional groups involved in the complexation of metal ions.

Key Words: *Rhizomucor tauricus*, Adsorption, Nickel, Experimental design, FTIR.

1. Introduction

Attention is primarily being given to the potential health hazard presented by heavy metals to the environment. Mining and metallurgical wastewaters are considered to be the major sources of heavy metal contamination, and the need for the removal of metals has resulted in the development of new separation technologies. Biosorption is being demonstrated to be a useful alternative to conventional systems like precipitation, ion exchange, electrochemical processes and membrane processes for the removal of toxic metals from industrial effluents [1]. This novel approach is competitive, effective and cheap [2. 3].

It has been reported that nickel is ubiquitous in the biosphere and is a common component of natural fresh waters due to erosion and weathering. The high consumption of nickel- containing products inevitably leads to environmental pollution by nickel and its by-products at all stages of production, recycling and disposal. It has also been reported that fresh water levels of Ni are typically about 1-10 µg/l in unimpacted areas and anthrogenic loading of Ni into aquatic and terrestrial ecosystems in industrial areas occurs by mining, smelting, refining, alloy processing, scrap metal reprocessing, fossil fuel combustion and waste incineration. Ni (II) concentrations in fresh waters may reach as high as several hundreds to 1000 µg/L. It is known, however, that exposure to nickel compounds can have adverse effects on human health [4, 5].

The immobilization of biomass might also provide several advantages such as facility to reuse and separation of solid biomass from the bulk liquid. The process will become cost effective by reusing the biomass after regeneration and immobilized fungal biomass i. e. white rot fungus *Trametes versicolor* [6], *Rhizopus arrhizus,* [7], *Phanerochaete chrysosporium* [8], *Trichoderma harzianum* [9] used to remove heavy metals from aqueous solutions. The immobilization method is easy and can be performed under very mild conditions without damaging the living fungal cells.

The present investigation was aimed at optimization of the nickel uptake form aqueous solution using immobilized *Rhizomucor tauricus*. The optimization of initial metal concentration, biomass loading and pH were carried out via Box-Behnken RSM experimental design. The interaction between factors influencing nickel uptake was established and model describing the effect of the factors on nickel uptake was also described.

2. Materials and Methods

2.1 Preparation of fungal beads

4g of sodium alginate was dissolved in 100ml hot water and stirred vigorously with magnetic stirrer for 10 minutes to obtain a thick uniform solution. Then sodium alginate solution was mixed with fungus, stirred vigorously for 15 minutes to get uniform suspension. This mixture was pumped through the peristaltic pump into the beaker containing 0.05M $CaCl_2$ $2H_2O$ solution. The spherical shaped beads with 4 mm diameter size were formed [10].

2.2. Equilibrium studies

100ml of Nickel solution of known concentration was charged into a 250 ml conical flask and immobilized beads were added. The biomass weight, pH and metal concentration were optimized by using the Box-Behnken design and analyzed for removal of Ni^{++} ions on immobilized *Rhizomucor tauricus*. The flasks were shaken for 4 hours using orbital shaking machine. The equilibrium time of about 4 hours was estimated from preliminary studies. The required pH was maintained and adjusted by adding 0. 1N hydrochloric acid and 0. 1 N sodium hydroxide to the aqueous solution. The samples were collected and analyzed for metal concentration using Atomic Absorption Spectrophotometer of Perkin-Elmer (model 3100) make.

At equilibrium the amount of metal uptake by *R.tauricus* was determined by using the following equation 1:

$$C_S = \frac{V(C_i - C_a)}{S} \qquad (1)$$

where C_S is the metal uptake of Ni (II) g^{-1} biomass, V is the volume of metal containing solution in contact with the biosorbent in mL, C_i and C_a are the initial and equilibrium (residual) concentration of metal in the solution mg L^{-1}, respectively, and S is the amount of added biosorbent on dry basis in g. The data were tabulated and applied Statistica version 6.0 for regression and graphical analysis.

2.3. Experimental design and optimization

R.S.M is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariable equation simultaneously. Box and Benkhen (1960) [11] have proposed some three-level designs for fitting response surfaces. These designs were formed by combining 2^k factorials with incomplete block designs. The resulting designs are usually very efficient in terms of the number of required runs, and they are either rotatable. Box-Benkhen designing (Montgomery, 2003) [12] is a spherical design with all points at the vertices of the cubic region created by the upper and lower limits for each variable. This could be advantageous when the points on the corners of the cube represent factor-level combinations that are prohibitively expensive of impossible to test because of physical process constrains.

Several experimental designs have been considered for studying such models and central composite design proposed by Adinarayana *et al.* (2003) [13] was selected. For this study, 3**(3-1) fractional factorial design with three replicates at the central points were employed to fit the second–order polynomial model, by using 'STATISTICA (Version 6.0)' software for regression and graphical analysis of the data obtained. Box-Benkhen design is used to determine the optimum conditions for the determination of Cs. For this experiment 3 factors with 12 runs are employed as in Table 2.

The parameters studied were pH, Concentration of Metal, and Beads volume Table 1. The variable levels X_i were coded as x_i according to the following equation such that X_0 corresponded to the central value

$$x_i = \frac{X_i - X_0}{\Delta X_i} \qquad i = 1, 2, 3.....k \qquad (2)$$

Where x_i is the dimensionless value of an independent variable Xi the real value of an independent variable, X_0 the real value of an independent variable at the central point and ΔX_i is the step change. The experimental plan and levels of independent variables are shown in Table 2. The response variable was fitted by a second order model in order to correlate the response variable to the independent variables. The general form of the second-degree polynomial equation is

$$Y_i = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i}^{i<} \sum_{j}^{j} \beta_{ij} x_i x_j + e ------ (3)$$

Where Y_i is the predicted response, x_i x_j are input variables which influence the response variable Y; β_0 is the off set term; β_i is the ith linear coefficient β_{ij} the ith quadratic coefficient. e is the error.

The statistical analysis of the model was performed in the form of analysis of variance (ANOVA). Where it was possible to simplify the model by dropping terms, which were not statistically significant (P>0.05) by ANOVA. The lack of fit test was used to determine whether the constructed model was adequate to describe the observed data. The R^2 statistic indicates the percentage of the variability of the optimization parameter that is explained by the model. Three-dimensional surface plots were drawn to illustrate the main and interactive effects of the independent variable on the dependent ones.

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Table 1 The experimental domain

Factor		Range and level (coded)		
		-1	0	1
Initial Metal Concentration	A	30	60	90
Volume of immobilized biomass	B	10	20	30
Initial pH	C	2	4	6

The optimum conditions for the adsorption of nickel by *R. tauricus* were determined by means of RSM. RSM consists of a group of empirical techniques devoted to the evaluation of relationships existing between a cluster of controlled experimental factors and measured responses according to one or more selected criteria [14]. In the present work effect of pH, biomass loading and initial nickel concentration were investigated. Each of the parameters was coded at three levels: -1, 0, 1, The range and the levels of the variables investigated in this research are given in Table 1.

3. Results and Discussiom

3.1. Equilibrium Studies

Time required to reach equilibrium was estimated by taking samples at regular intervals of time and it was found to be 4 hours. Hence, all the experimental runs were conducted for 5 hours in order to ascertain the attainment of equilibrium and the variation of metal concentration, in the solid phase was compared with that in aqueous phase using alginate beads without biomass and immobilized biomass.

3. 2. Interpretation of regression analysis

The results obtained after running the 12 trials for the statistical design shown in Table 2. The actual yields of Cs in the experiments and the yield predicted by the model are given in the Table 3. The regression coefficients and significance levels of the terms are given in Table 4. The fit of the model was checked by the determination of the coefficient of regression (R^2). In this case the value of the determination coefficient (R^2= 0.9921) indicates that only 1.6% of the total variation is not explained by the model. The value of the adjusted determination coefficient (adjusted R^2 = 0.9827) is high, indicating the significance of the model. A higher value of the correlation coefficient (0.99217) justifies an excellent correlation between the independent variables as shown Table 5. The same results found by other workers in Box–Behnken model with *Phanerochaete chrysosporium* immobilized Ca-alginate beads as indicated by the correlation coefficient value of 0.9999 [15].

Table 2. The experimental Design with coded values

S. No.	Concentration	pH	Biomass Volume
1	0	0	0
2	0	0	20
3	-1	1	0
4	-1	-1	-1
5	0	0	0
6	0	1	-1
7	1	0	-1
8	0	0	0
9	1	1	1
10	0	-1	1
11	-1	0	1
12	1	-1	0

ISSN: 2248-9622 www.ijera.com Vol. 2, Issue 3, May-Jun 2012, pp.2810-2819

Table 3. The experimental design with real values, C_S observed and predicted.

S. No.	Conc. mg/L X_1	PH X_2	B. Volume ml X_3	Observed C_S	Predicted C_S	Error
1	60	4	20	446	421.8	24.2
2	60	4	20	395	421.8	-26.8
3	30	6	20	340	343.4	-3.4
4	30	2	10	71	69.2	1.8
5	60	4	20	420	421.8	-1.8
6	60	6	10	433	431.4	1.6
7	90	4	10	289	292.4	-3.4
8	60	4	20	428	421.8	6.2
9	90	6	30	311	309.2	1.8
10	60	2	30	161	164.4	-3.4
11	30	4	30	189	187.4	1.6
12	60	2	20	183	181.4	1.6

3.3. Fitting the model

The application of R.S.M. yielded the following regression equation, which is an empirical relationship between the Cs and test variables.

$Y = 421.8+30.5\,X_1-108.7X_1^2 + 111.5X_2 --50.70X_2^2\ -22.00X_3 -73.2X_3^2 + e$ (4)

The significance of each coefficient was determined by student's t-test and P-Value, which are compiled in Table 4. The larger the t-value and smaller the P-value, the more significant is the corresponding coefficient. It is also observed that from Pareto chart of effects the variables (Fig.1) that are crossing line are significant. This implies that all the variables are more significant at the quadratic terms than the linear terms. Only the linear term of concentration is insignificant and all interaction terms are insignificant. From this observation the regression equation is reduced to.

$Y = 421.8-108.7X_1^2 + 111.5X_2 -50.70X_2^2 -22.0X_3\ -73.20X_3^2$ (5)

All the variables are more significant at the second order. This indicates that they can behave as limiting factors and small variations will alter the product formation rate.

Table 4. Regression coefficients

	Regression Coefficients	Standard Error	t-Value	p-Value
Intercept	421.8	8.066	52.288	0.000000
C_T (L)	30.50	6.817	4.4737	0.006557
C_T (Q)	-108.7	10.561	-10.291	0.000149
pH (L)	111.5	6.871	16.354	0.000016
pH (Q)	-50.70	10.561	-4.8003	0.004882
B.V (L)	-22.00	6.817	-3.2269	0.023285
B.V (Q)	-73.20	10.561	-6.9306	0.000960

L=Linear, Q=Quadratic

Table 5. Analysis of variance for the quadratic model for Ni^{++} C_S.

	SS	df	MS	F	P
C_T (L)	5581.5	1	5581.50	20.0140	0.006557
C_T (Q)	29539.2	1	29539.23	105.9209	0.000149
pH (L)	74593.5	1	74593.50	267.4753	0.000016
pH (Q)	6426.2	1	6426.23	23.0430	0.004882
B.V (L)	2904.0	1	2904.00	10.4131	0.023285
B.V (Q)	13395.6	1	13395.60	48.0336	0.000960
Error	1394.4	5	278.88		
Total SS	178065.0	11			

L=Linear, Q=Quadratic

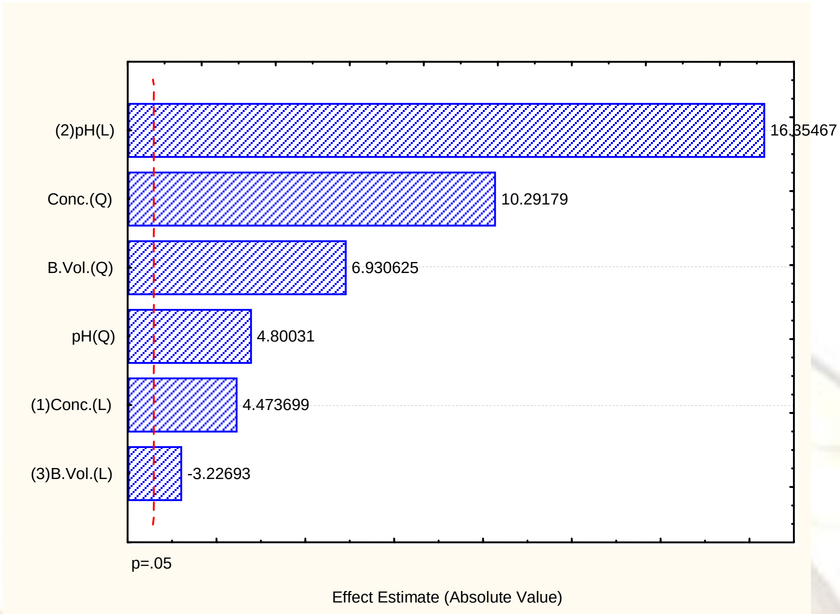

Figure 1 Pareto Chart of effects on metal concentration on solid biomass C_S

3. 4. Interpretation of Surface plots for Ni (II) removal

The response surfaces are inverted paraboloids and they can be used to predict the Cs for different values of the test variables and to identify the major interactions between the test variables from the circular or elliptical nature of surface contours (Figure 2, 3 & 4). These plots can easily be obtained by calculating the values taken by one factor while the second varied with constraint of a given C_S value. The yield value for different concentration of the variables can also be predicted from the respective contour plots. The stationary point or centroid point considering all the directions, is the point at which the slope of the response surface is zero. Experiments were performed beyond the experimental range to confirm the presence of optimum points.

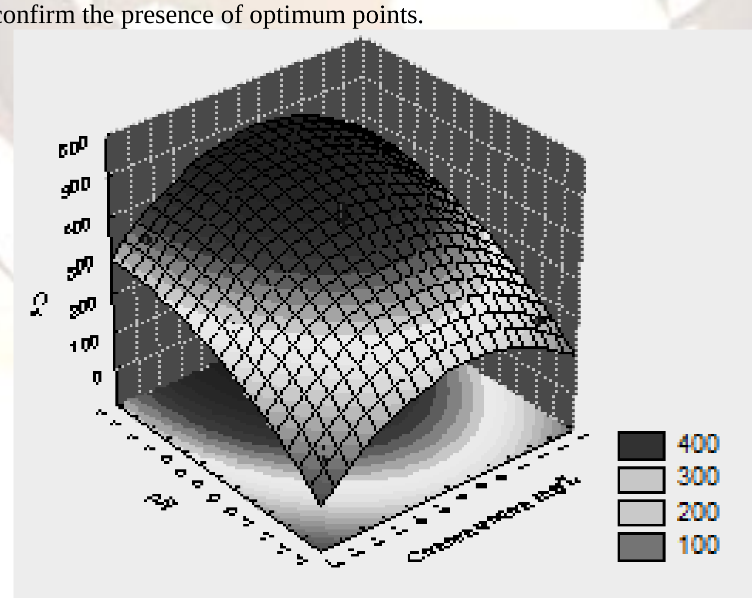

Figure 2 Response surface plot of C_S at various pH and biomass volume

In view of the above and inference can be drawn that the factors influenced the maximum C_S are the pH 6.0, concentration is 60 mg L^{-1}, biomass volume is 20 ml/100ml (6 g L^{-1} of biomass)**.** In case of fungus *Aspergillus niger*,

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the optimum Ni (II) uptake was achieved at pH 6.25, biomass dosage of 2.98 g L^{-1} and initial Ni(II) concentration of 30.00 mg L^{-1} Ni (II) [16]

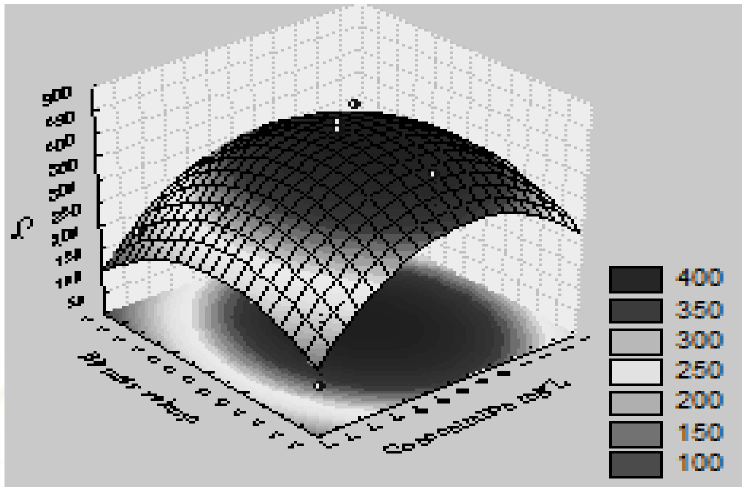

Figure 3 Response surface plot C_S at various biomass volume and initial metal concentration

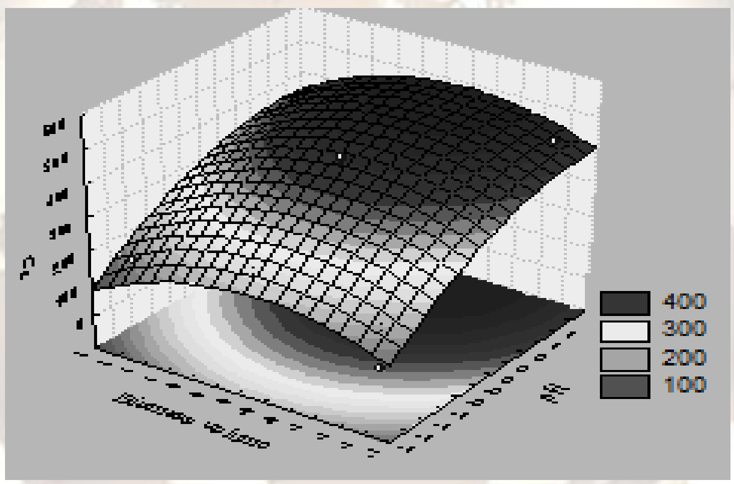

Figure 4 Response surface plot of C_S at various biomass volumes and pH

By observing the marginal means of the individual variables, it was found that the concentration is more significant at 60mg/L (fig. 5) positive or negative deviation results the decrease the Cs, the reduction of the Cs is more on positive deviation side. Fig. 6 shows pH is more significant at 6, either increasing or decreasing the pH resulted in a fall of Cs. The same results were obtained on fungal mycelium of *Rhizopus arrizhus* Fourest and Roux (1992) [17] and Sag *et al* (1995) [18]. This is probably due to preferential adsorption of acid by immobilized biomass. This behavior facilitates the desorption of metal from the solid surface.

ISSN: 2248-9622 www.ijera.com Vol. 2, Issue 3, May-Jun 2012, pp.2810-2819

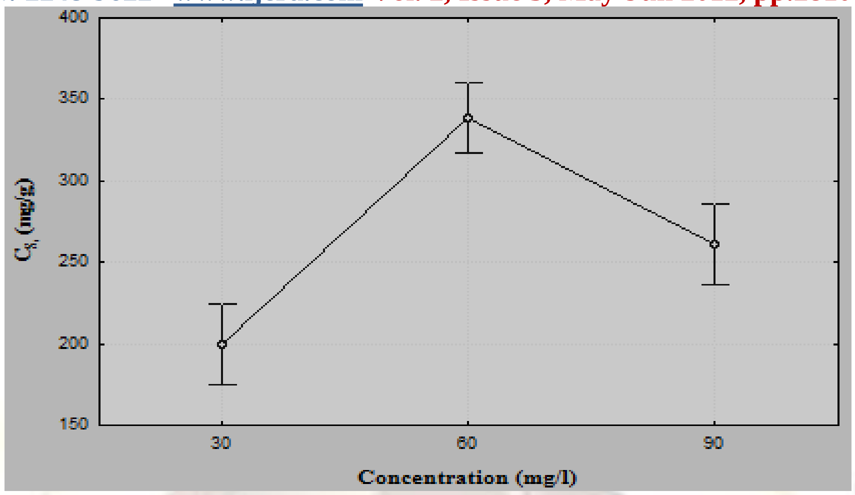

Figure 5 Effect of initial metal concentration on uptake efficiency of biomass beads

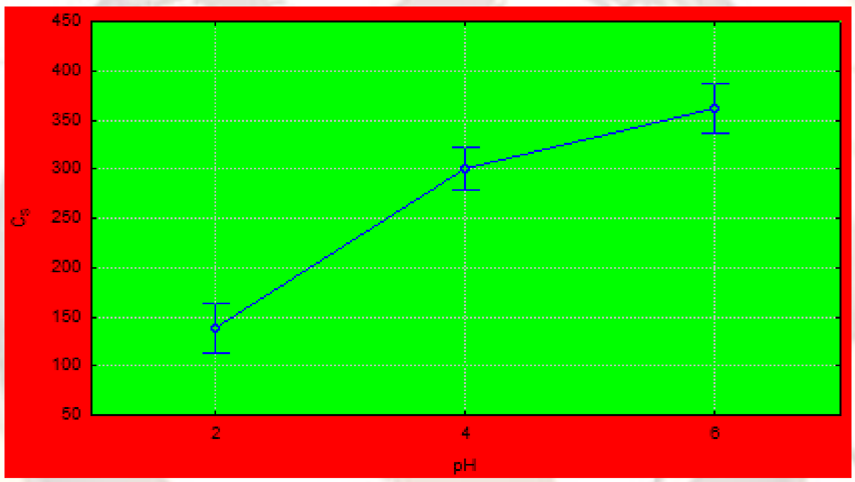

Figure 6 Effect of pH on uptake efficiency of biomass beads

Fig. 7 shows that the Biomass volume is more significant at 20 ml/100 ml, further increase in the volume of biomass beads decreased metal adsorption. This may be attributed to the overlapping [19] of the biomass in the beads as well as collision in between the beads in the agitation. When an optimization program was run within the tested range the optimum level of process variables could be summarized as Concentration 60mg/L, pH 6, Biomass volume 20 ml/100 ml and with these levels the model has predicted yield of 425mg/g, which is nearer to the observed values. These results indicate that there is a very good correlation (Fig. 8) between the experimental values and the predicted values and also indicate that the experimental error was found to be minimum.

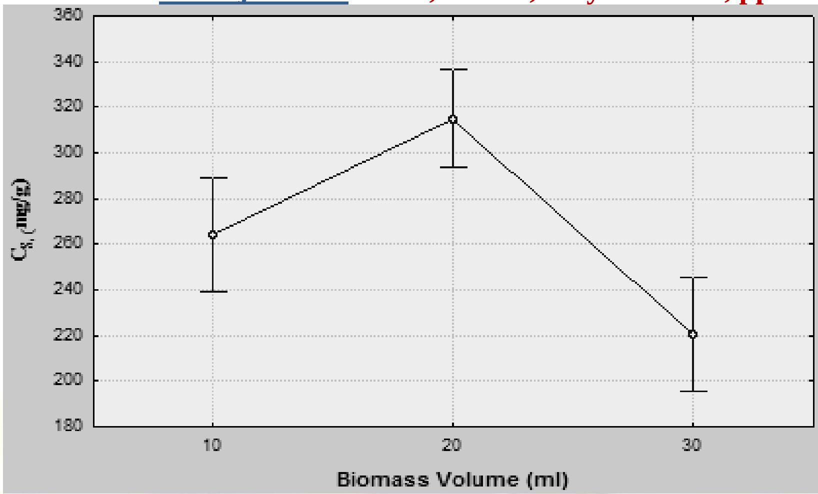

Figure 7 Effect of biomass volume on uptake efficiency of biomass beads

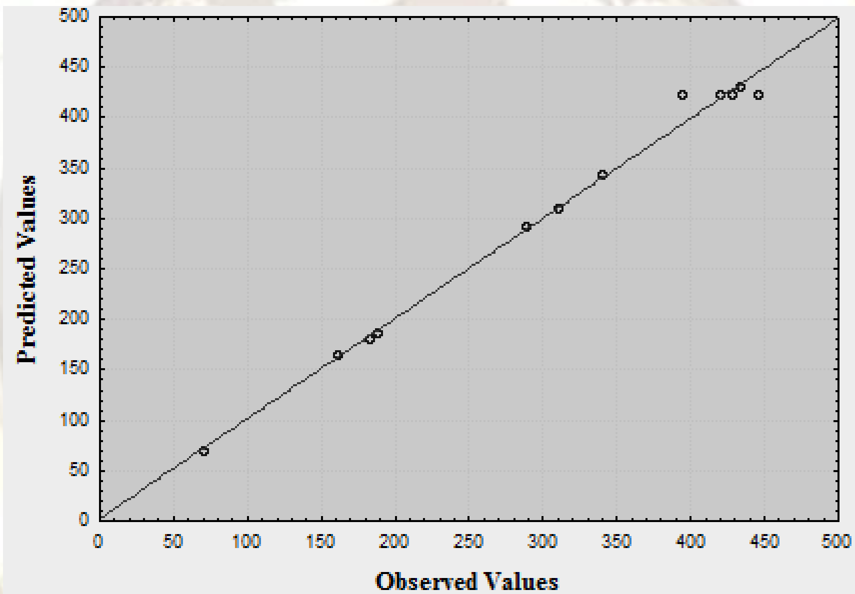

Figure 8 observed and predicted values

3.5. FTIR Spectroscopic Analysis

The FTIR spectroscopic analysis of the biomass, dead powder of *R. tauricus* before adsorption of heavy metal ions (Fig. 9), indicated a broad adsorption band at 3423.28 Cm^{-1}, representing –NH stretching [20, 21] (Singh *et al*. 2010; Venkata Subbaiah *et al*. 2010); 2925.21 Cm^{-1}, and 2854.16 Cm^{-1} represented $–CH_2$ stretching. The absorption band at 1742.68 Cm^{-1} could be attributed to a C=O group of carboxylic acid [22], and the absorption band at 1417.14 Cm^{-1} represents a carboxylate group. Further, the band at 1180.53 Cm^{-1} indicate an –OH group of sugars, and the bands at 1076.53 Cm^{-1} and 1031.88 Cm^{-1} represent amide C-N stretching and –P=O stretching, respectively.

ISSN: 2248-9622 www.ijera.com Vol. 2, Issue 3, May-Jun 2012, pp.2810-2819

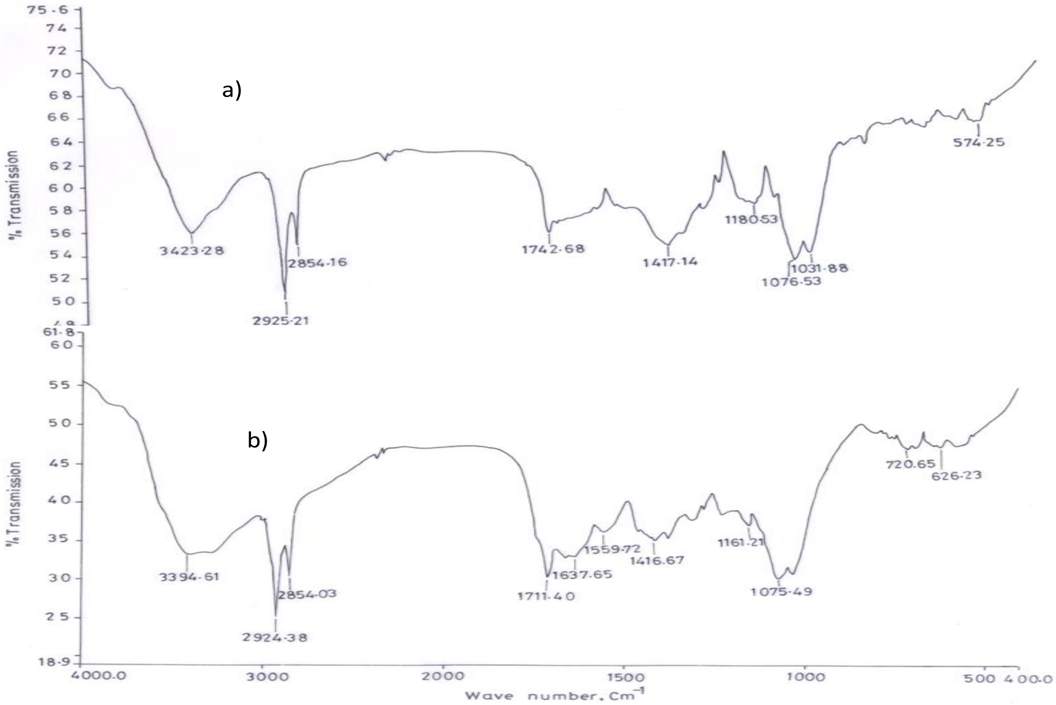

Figure 9 FTIR of *Rhizomucor tauricus* a) before treatment, b) after treatment with Ni (II)

In all the FTIR spectroscopic analysis of the biomass, dead powder of *R. tauricus* after adsorption of heavy metal ions (Fig. 9) revealed that the shift of peaks at 3423.23 Cm^{-1}, 1742.68 Cm^{-1}, and 1180.53 Cm^{-1} indicated that the amide – NH bending, CH stretching, carboxylic acid, and hydroxylic groups are the main functional groups involved in the complexation of metal ions.

4. Conclusions

The response surface methodology optimization revealed that the tested range the optimum level of process variables were Concentration is 60 mg/L, pH is 6, biomass volume was 20 and with these levels the model has predicted yield 449 mg/g, this value is nearer to the observed values. FTIR spectral analysis indicated that the amide – NH bending, CH stretching, carboxylic acid, and hydroxylic groups are the main functional groups involved in the complexation of metal ions. These results indicate that there is a very good correlation between the experimental values to predicted values and also indicate there is a minimum experimental error is taking place. This data revealed that the immobilized biomass beads adsorbed more metal when compared to blank alginate beads. *Rhizomucor tauricus* may be used as efficient adsorbent to remove nickel (II) from effluents.

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