



## **Cost effective and eco-friendly method for copper removal by adsorption with Emu feather (*Dromaius novaehollandiae*) and Chitosan (*Agaricus bisporus*) Composite**

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**Abstract:** Adsorption of copper ions from aqueous solution onto the composite biomaterial made of *Dromaius novaehollandiae* feathers (DNF) and Chitosan (CH) has been investigated to evaluate the effects of contact time, pH, initial adsorbate concentration, adsorbent dosage and temperature by one variable at a time method initially followed by a three level full factorial design of Response Surface Methodology (RSM) for understanding the interactive effects of operating parameters. Kinetic, equilibrium and thermodynamic calculation results showed that the kinetics followed pseudo second order, equilibrium models fitted to Langmuir and Freundlich, and thermodynamic parameters indicated that the process was spontaneous, irreversible and endothermic in nature. From the results of RSM, optimum values for adsorptive removal of copper by the DNF-CH composite were determined as 6.93 g/L of adsorbent dosage, 19.7748 mg/L of initial adsorbate concentration and a neutral pH of 7. Maximum percentage of copper adsorption was found to be 93.91% (18.78mg L<sup>-1</sup>). The adsorbent was characterized before and after adsorption by SEM – EDS, FTIR and XRD.

**Key words :** Adsorption, *Dromaius novaehollandiae* feathers (DNF), Chitosan (CH), DNF-CH composite, full factorial design, copper.

### **Introduction**

Among all environmental pollutions, water pollution by the industrial waste which consists of toxic heavy metals is serious. Hazardous heavy metals present in industrial wastes are copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), chromium (Cr), mercury (Hg) and others. Heavy metals are toxic in nature because of their accumulation in living tissues and environment. Metal cleaning and plating baths, pulp, paper board mills, printed circuit board production, wood pulp production, fertilizer industry etc. are releasing copper into the environment<sup>1</sup>. Copper and its compounds are ubiquitous in nature and will bind to natural organic materials and soil particles, and hence commonly found in surface water. An ultra trace amount of copper is essential for living organisms. According to Safe Drinking Water act, copper has a permissible limit of 1.3mg/L in drinking water<sup>2</sup>. Beyond these levels, in human beings it causes stomach upset and ulcer, mental retardation, liver and brain damage and so on<sup>1</sup>. Therefore the safe and effective removal of copper from contaminated fresh waters is a significant environmental issue of global concern.

Many conventional methods have been developed for the removal of heavy metals from effluents such as sedimentation, ion exchange, membrane filtration, electrochemical processes, chemical precipitation, reverse

osmosis and solvent extraction. But these methods are expensive or inefficient when the concentrations of metals are low (below 100ppm) and there is generation of large quantities of wastes. Development of eco-friendly, efficient and low - cost processes is the need of the hour and in this aspect, adsorption is a versatile technology with the advantages of high efficiency and selectivity for adsorbing metals in low concentrations, recycling of the adsorbent and minimization of the sludge generation.

### **The adsorbents may be either natural (organic or biological origin) or synthetic polymers.**

During the last decade several natural adsorbents like microorganisms<sup>4-9</sup>, agricultural wastes<sup>10-13</sup>, poultry wastes<sup>14-17</sup>, marine wastes<sup>18-20</sup>, several synthetic polymer composites<sup>21-27</sup> etc. are effectively used for the removal of heavy metals. The main governing factors in selecting a suitable adsorbent are abundant, renewable and biodegradable resources with a capacity to associate with a wide variety of molecules by physical and chemical interactions. In compliance with the said factors, in the present study, eco friendly and abundantly available poultry waste material namely feathers (F) of *Dromaius novaehollandiae* were used to synthesize DNF - Chitosan (CH) composite as an adsorbent to remove copper from aqueous solutions. Feathers are non-abrasive, low density, good mechanical properties, insoluble in water and organic solvents at ambient temperatures but solubility is enhanced with rise in temperature and addition of hydrophobic groups in the solvents. These properties of feathers are useful in making the polymer composite with chitosan. Keratin from chick feathers and wool has been used by previous researchers in making composites due to its importance in textile, cell cultivation and medicine<sup>28-31</sup>. Emu feathers consist of a fibrillar protein, keratin made of different kinds of amino acids with the reactive functional side chains arranged in twisted  $\beta$ -sheets<sup>32</sup>. This property could be used in environmental decontamination, especially for the removal of heavy metals. The emu feathers (DNF) and the soft gel forming chitosan (2-acetamido-2-deoxy- $\beta$ -D-glucose-N acetylglucosamine) processed from white mushroom, *Agaricus bisporus*, were combined in the ratio of 5:1 to get DNF - CH composite. Feather fiber provides a strong support to the Chitosan, so that the surface area of the chitosan increases. The objective of the present work is to know the adsorption kinetics, adsorption isotherms, thermodynamics and finally understanding the copper adsorption mechanism through Scanning Electron Microscopy (SEM)- Energy dispersive spectroscopy (EDS), Fourier Transform Infra Red spectroscopy (FTIR) & X-Ray Diffraction (XRD). To study the cumulative / interactive effects and optimization of copper adsorption process, Full Factorial Design of RSM was applied, with three independent parameters at three levels. Full Factorial design is the simplest one to optimize three variables. In this design maximum information regarding the factors is obtained by trying all possible factor level combinations.

## **2. Experimental**

### **Adsorbents**

Feathers of *Dromaius novaehollandiae* (DNF) were collected from poultry processing facilities of Bapatla, Andhra Pradesh, India. Feathers were washed several times with deionized water to remove dirt particles, the barbs were detached from the shaft and used for the adsorption studies. Chitosan (Prepared from *Agaricus bisporus*) was purchased from Sigma Aldrich (740179). Prior to composite preparation, feathers were treated with 2% aqueous acetic acid and neutralized with 2% NaHCO<sub>3</sub>. Chitosan was dissolved in 10% aqueous acetic acid and to it, added the neutralized DNF solution. Then the mixture was dropped into an alkaline coagulant solution (H<sub>2</sub>O: MeOH: NaOH = 5:4:1 w/w). The DNF-CH (5:1) composite was then washed with distilled water.

### **Adsorbate**

Stock solution was prepared by dissolving 3.798g of Cu(NO<sub>3</sub>)<sub>2</sub>.3H<sub>2</sub>O in 250ml of deionized water, diluted to 1 liter in a volumetric flask with double distilled water procured from Millipore ELIX-10 unit. Test solutions were prepared by progressive dilution of stock solution of copper with double distilled water and the pH was adjusted to the appropriate value by using 0.1N HNO<sub>3</sub> or 0.1N NaOH solutions. All chemicals used in the present study were of analytical grade.

### **Batch adsorption**

Adsorption was carried out in a batch process using one variable at a time by contacting 300 mg of DNF-CH composite with 50ml of 20mg/L of copper solution in 250ml Erlenmeyer flasks; then the flasks were agitated on an orbital shaker (REMI make CIS-24BL) at 180rpm and 30<sup>o</sup>C temperature. Samples were taken at

predetermined time intervals of 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 min. For further experiments, contact time was adjusted to the optimum. Kinetic models including pseudo-first-order and pseudo-second-order were used to determine the rate of adsorption of copper. The effect of pH was studied in the range of 2 - 9. With optimum contact time and pH, metal solutions in the concentration range of 20 to 100mg/L were used to assess the effect of initial copper ion concentrations. The sorption equilibrium between the adsorbent and metal ions was determined by using Langmuir, Freundlich and Temkin models. For all the three models, the isotherm constants were obtained by non-linear regression methods. Similarly, the adsorbent dose was varied from 150 mg to 450 mg and finally temperature was varied between 10<sup>0</sup>C and 50<sup>0</sup>C. Thermodynamic parameters were calculated using Van't Hoff equation. All the experiments were carried out in duplicate and the average values are reported. At the end of each adsorption process, the adsorbate was filtered out through whatman filter paper and the residual metal concentration was determined by Atomic Absorption Spectrophotometer (Shimadzu make AA-6300) with copper hollow cathode lamp using air acetylene flame at a wavelength of 324.8nm. The percentage removal was obtained by using the expression

$$\text{Percentage removal (\%)} \text{ of metal} = [(C_o - C_e)/C_o] \times 100 \quad (1)$$

where  $C_o$  is the initial concentration of stock sample (mg/L),  $C_e$  is the final concentration of stock sample after adsorption (mg/L).

### Adsorption optimization and Statistical analysis

Design expert 9 (Stat-Ease Inc., Minneapolis, MN, USA) software trial version was used for three level full factorial design of RSM to optimize the selected design variables<sup>33,34</sup> for the maximum adsorption of copper by DNF-CH composite. A total of 28 experimental runs were conducted to describe the effects of all the three variables ( $X_1$  = adsorbent dosage,  $X_2$  = initial metal concentration,  $X_3$ = initial pH values) on adsorption. The coded values of the process parameters were determined by the equation

$$x_i = \frac{X_i - X_o}{\Delta X} \quad (2)$$

Where  $x_i$  is the coded value of the  $i^{\text{th}}$  variable,  $X_i$  is the uncoded value of the  $i^{\text{th}}$  test variable and  $X_o$  is the uncoded value of the  $i^{\text{th}}$  test variable at the center point. The levels and ranges of the coded variables are given in Table 1. The behaviour of the experimental design is explained by the following polynomial second order equation

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

Regression equation was solved using fmincon function of Matlab 2008 and with the optimum values obtained, surface plots were drawn.

**Table 1. Experimental ranges and levels of independent variables for adsorption of copper**

Variables	Coded Values		
	-1	0	+1
Biosorbent Dosage, w, g/L ( $X_1$ )	6	7	8
Initial metal concentration, $C_o$ , mg/L ( $X_2$ )	15	20	25
pH of aqueous solution ( $X_3$ )	5	6	7

### Analysis of adsorption mechanism

In order to observe the surface changes of the adsorbent after sorption of metal ions and *in situ* metal analysis, SEM (EVO 18 make Carl Zeiss) and EDS (Oxford instrument, Inca) analysis were employed respectively. To know the main functional groups present on the adsorbent and their interaction with the metal ions, Fourier transform Infrared (Bruker U.K, ATR) analysis was performed. The adsorbent (before and after sorption) was characterized by X-ray diffraction (XRD) technique using X-ray diffractometer with CuK $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ). The measurement was in the scanning range of 5-100 at a scanning speed of >19.685s<sup>-1</sup>.

## Results and Discussion

### Effect of contact time

The results showed that the percentage removal of copper increased rapidly up to 70 min reaching 89.875% (Figure 1), due to the availability of adequate vacant surface binding sites especially hydroxyl and amino groups present on the DNF - CH composite. Beyond 70 min, the % of adsorption remained constant indicating the attainment of equilibrium conditions, due to repulsive forces between the solute molecules of the solid and the bulk phases; consequently, the remaining vacant binding sites failed to bind with the metals<sup>34</sup>.

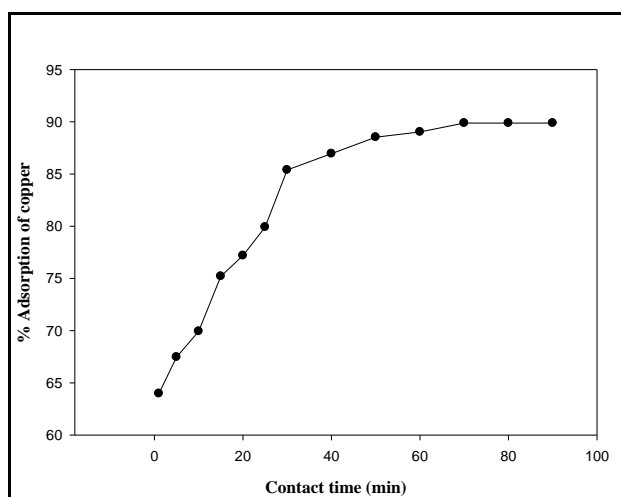


Figure 1. Effect of contact time on % adsorption of copper

### Effect of pH on adsorption

One of the most significant factors influencing adsorption is the pH as it affects the adsorbent surface charge, the degree of ionization and speciation of the adsorbate as well. Maximum % removal of copper was 91.41% at pH 6 (Figure 2) and this is in agreement with the earlier research reports<sup>35,36</sup>. The increase in adsorption at pH 6 could be attributed to the weak inhibitory effect of H<sup>+</sup> ions and consequent replacement of the H<sup>+</sup> ions bound to the adsorbent by the copper ions. At pH <5, the number of sites available for metal adsorption will be low as most of the functional groups are protonated and H<sup>+</sup> ions compete with the metal ions for the adsorption sites of the adsorbents.

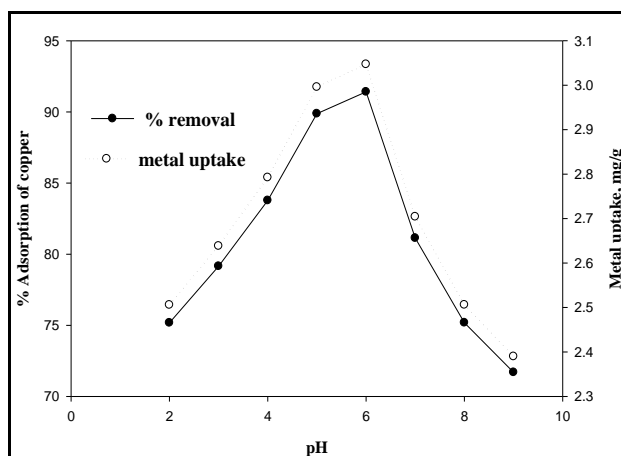


Figure 2. Effect of pH on % adsorption of copper

### Effect of initial metal ion concentration

The maximum percentage removal of copper observed was 91.41% when the initial copper concentration was 20mg/L (Figure 3). The plot suggests that the metal ion adsorption increased in the beginning and then slowly decreased at the end. This can be attributed to the increase in adsorbate concentration to a fixed number of available active sites on the adsorbent<sup>37</sup>.

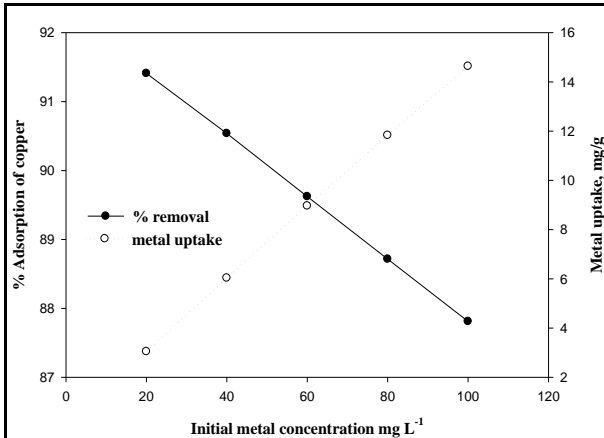


Figure 3. Variation of initial metal concentration with % adsorption of copper

**Effect of adsorbent dosage**

The percentage removal of copper by DNF-CH composite at different adsorbent doses is presented in Figure 4. Experimental studies were carried out at 30<sup>0</sup>C temperature with an initial metal concentration of 20mg/L at pH 6. The removal of copper increased rapidly from 87.215% to 93.105% with an increase in adsorbent dose from 3 to 7g/L, due to the increased surface area of the adsorbent and the number of binding sites. With further increase in the adsorbent dosage, the percentage of adsorption remained constant. This is obvious because for a fixed initial metal concentration, although the adsorbent dose was increased, there would be no significant change in the adsorption after attaining the equilibrium<sup>38,39</sup>.

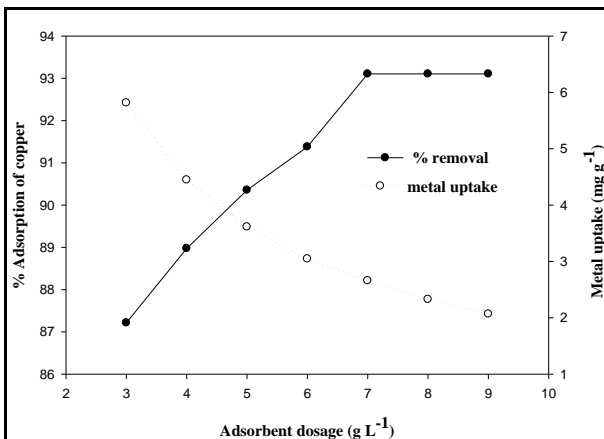


Figure 4. Dependency of % adsorption of copper on adsorbent dosage

**Effect of temperature**

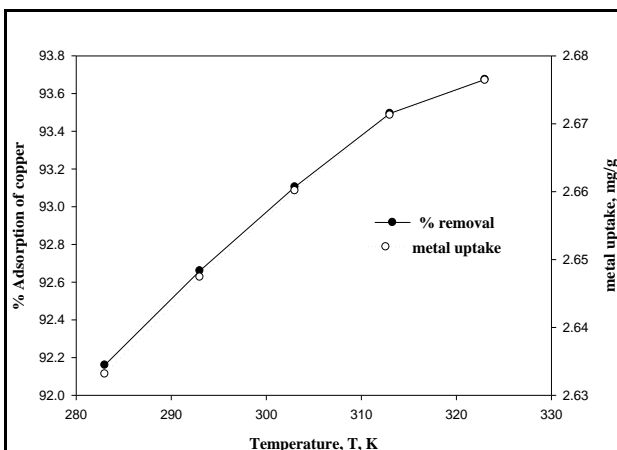


Figure 5. Dependency of % adsorption of copper on temperature

Copper uptake marginally increased from 92.16% to 93.105% for DNF-CH composite with increasing temperature from 10°C to 30°C indicating that the adsorption of copper on to the adsorbent is an endothermic process. When the temperature was lower than 30°C, copper uptake increased with increase in temperature, but when the temperature was above 30°C, the increase in uptake was marginal (Figure 5). Adsorption was expected to increase by an increase in temperature owing to the increase in the rate of diffusion of the adsorbate molecules across the external boundary layer and into the internal pores of the adsorbent particles.

### Adsorption Kinetics

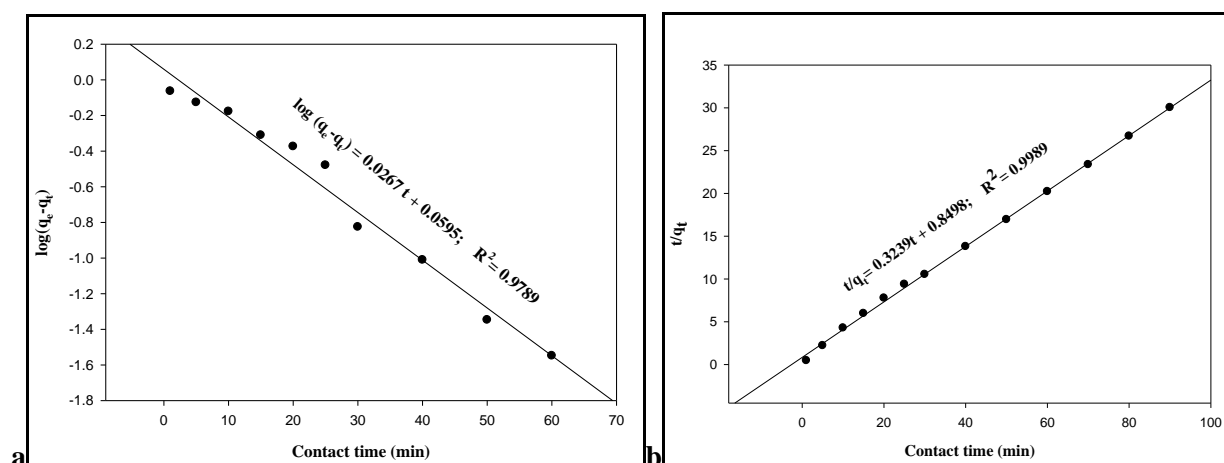
The kinetics of adsorption of copper at different contact times was found out using the pseudo - first order and the pseudo - second order models. The pseudo – first order equation is

$$\log (q_e - q_t) = \log q_e - K_1/2.303 (t) \quad (4)$$

where  $q_e$  and  $q_t$  are the amount of metal adsorbed at equilibrium and at time  $t$ ;  $K_1$  is the rate constant of pseudo – first order ( $\text{min}^{-1}$ ).

The pseudo – second order equation is  $t/q_t = 1/K_2 q_{\text{max}}^2 + 1/q_{\text{max}} (t)$  (5)

where  $K_2$  ( $\text{min}^{-1}$ ) is the pseudo - second order rate constant and  $q_{\text{max}}$  is the adsorption capacity. The pseudo - first and the pseudo - second order kinetic plots are given in Figures 6a and 6b respectively. In the pseudo - first order the  $K_1$  and  $q_e$  values are 0.0614 and 1.1468 respectively with the correlation coefficient ( $R^2$ ) of 0.9789. In the pseudo - second order the  $K_2$  and  $q_e$  values are 0.1234 and 3.0873 respectively with the correlation coefficient ( $R^2$ ) of 0.9989. This suggests that the adsorption of copper by DNF-CH composite preferably follows the pseudo- second order model. The confirmation of pseudo - second order kinetics indicates that in the adsorption process, both the adsorbent and the adsorbate concentrations are involved in the rate determining step.



**Figure 6 Kinetics for adsorption of copper using DNF-CH Composite a. Pseudo – first order b. Pseudo – second order kinetics**

### Adsorption equilibrium isotherms

The experimental data were tested and compared with three isotherm models viz. Langmuir, Freundlich and Temkin. The isothermal constants are presented in Table 2. Langmuir isotherm, the most widely used model, is given as  $(c_e/q_e) = 1/(bq_m) + c_e/q_m$  (6)

Langmuir isotherm drawn for our data (Figure 7a) indicates strong binding of copper ions on to the surface of the adsorbent. Freundlich isotherm, applied in the cases of low and intermediate concentration ranges, is given as

$$q_e = K_f C_e^n \quad (7)$$

where  $K_f$  and  $n$  are adsorption capacity and intensity respectively. Freundlich equation can be linearized in logarithmic form as

$$\log q_e = \log K_f + n \log C_e \quad (8)$$

With the experimental data represented in Figure 7b, the adsorption appears to be favored over the entire concentration range. Temkin isotherm equation describes the behavior of many adsorption systems on the heterogeneous surface. The linear form of the equation is

$$q_e = (RT/b_T) \ln (A_T) + (RT/b_T) \ln (c_e) \quad (9)$$

The experimental data were analyzed according to the equation and depicted in Figure 7c. Based on the linear regression correlation coefficient  $R^2$ , the Langmuir and the Freundlich isotherms were inferred to be the best fitted models followed by the Temkin model.

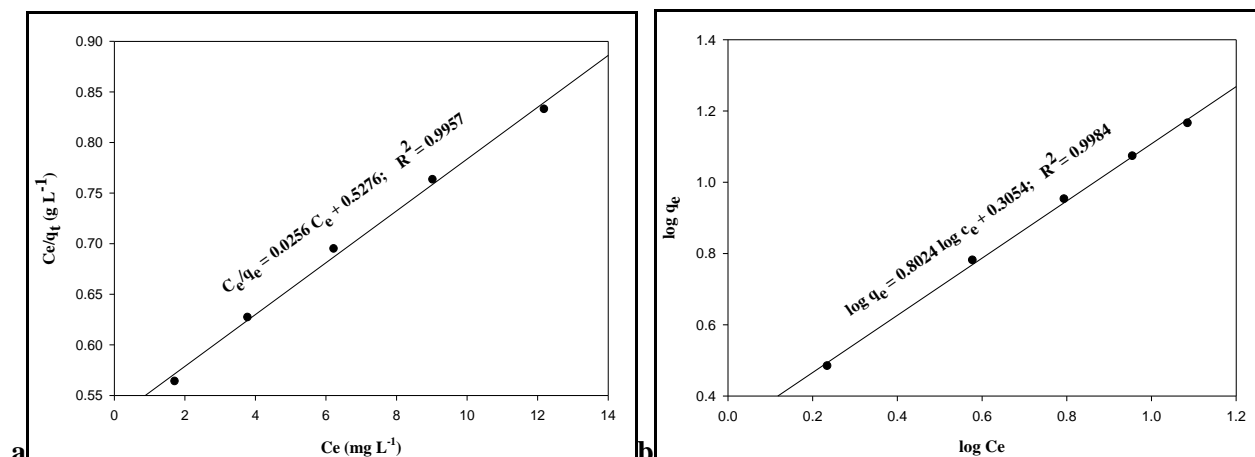


Figure 7 Equilibrium isotherms for adsorption of copper using DNF-CH Composite a. Langmuir b. Freundlich

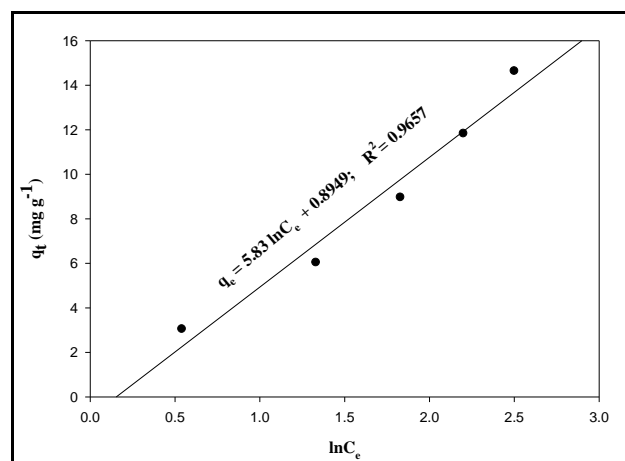


Figure 7c. Temkin isotherm for adsorption of copper using DNF-CH Composite

Table 2. Isothermal constants for the Langmuir, freundlich and temkin models

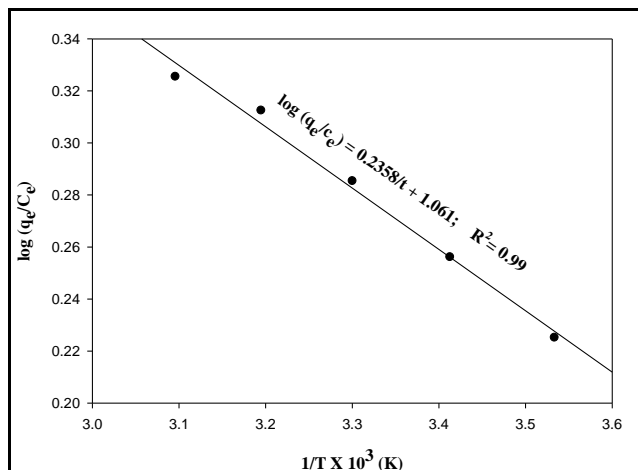
Langmuir	Freundlich	Temkin
$q_{max}=39.06 \text{ mg/g}$	$n=0.8024$	$A_T=0.8577$
$R_L=0.9236$	$K_f=2.0202 \text{ mg/g}$	$b_T =432.099$

### Thermodynamic Studies

To investigate the nature of adsorption, the three thermodynamic parameters, enthalpy ( $\Delta H^\circ$ ), entropy ( $\Delta S^\circ$ ) and Gibbs free energy ( $\Delta G^\circ$ ), were estimated. The following Van't Hoff equation was used to evaluate thermodynamic parameters:

$$\log (q_e/c_e) = \Delta H/2.303RT + \Delta S/2.303R \quad (10)$$

Van't Hoff plot is represented in Figure 8 and the equation obtained was  $\log (q_e/c_e) = 0.2358(1/T) + 1.061$ . The experimental  $\Delta H^\circ$  value of  $4.5148 \text{ kJ mol}^{-1}$  indicates that the adsorption process was endothermic in nature and there was a possible strong bonding between the metal ion and the adsorbent. As  $\Delta S^\circ$  ( $20.3151 \text{ kJ mol K}^{-1}$ ) was more than zero, the adsorption process appears to be irreversible. The Gibbs free energy ( $-6150.9605 \text{ kJ mol}^{-1}$ ) is negative, suggesting the spontaneous nature of the adsorption process. The free energy change ( $\Delta G^\circ$ ) increased with increase in temperature ( $10^\circ\text{C} - 50^\circ\text{C}$ ), possibly due to activation of more sites on the surface of the adsorbent<sup>31</sup>.



**Figure 8. Van't Hoff plot for biosorption of copper using DNF-CH composite**

Adsorption capacities ( $q_{\text{max}}$ ) of a few other reported adsorbents such as *Pseudomonas aeruginosa*<sup>40</sup>, saw dust<sup>41</sup>, wheat bran<sup>42</sup>, chitosan supported on porous glass beads<sup>22</sup>, n-HAp, n-HAp/chitin (n-HApC) composite and n-HAp/chitosan (n-HApCs) composite<sup>27</sup> for copper were  $5.83 \text{ mg/g}$ ,  $4.9 \text{ mg/g}$ ,  $8.62 \text{ mg/g}$ ,  $7.27 \text{ mg/g}$ ,  $4.7 \text{ mg/g}$ ,  $5.4 \text{ mg/g}$  and  $6.2 \text{ mg/g}$  respectively, revealing that the DNF-CH composite with a  $q_{\text{max}}$  of  $39.06 \text{ mg/g}$  is much effective and efficient for the adsorption of copper.

**Statistical analysis**

With the preliminary batch experiments, highest adsorption capacity was obtained at an adsorbent dosage of  $7 \text{ g/L}$ , initial metal ion concentration of  $20 \text{ mg/L}$ , and  $\text{pH } 6$ . The coded and the actual values of the test variables are as given in Table 3. Multiple regression analysis of the data for adsorption (Equation 11) yielded the following regression equation.

$$Y = +93.91 - 0.19 X_1 - 0.016 X_2 - 0.12 X_3 - 1.59 X_1 X_2 - 0.62 X_1 X_3 - 0.82 X_2 X_3 - 3.11 X_1^2 - 1.47 X_2^2 + 0.26 X_3^2 \quad (11)$$

Where Y is the % adsorption of copper,  $X_1$  adsorbent dosage,  $X_2$  is the metal ion concentration and  $X_3$  is the pH. Solving the regression equation using fmincon function, the optimum set of values for the three variable  $X_1$ ,  $X_2$  and  $X_3$  were  $-0.0668$ ,  $-0.2482$  and  $1.0000$ . Hence it was inferred that for maximum adsorption with the chosen adsorbent dosage, metal ion concentration and pH should ideally be kept at  $6.93 \text{ g/L}$ ,  $19.7748 \text{ mg/L}$  and  $7$  respectively.

**Table 3. Full Factorial design for adsorption of copper by DNF-CH composite**

Run No.	Coded Values			% of Adsorption		Residual	% Error
	$X_1$	$X_2$	$X_3$	Actual	Predicted		
1	6	15	5	87.28	86.88	0.40	0.45
2	7	15	5	91.58	92.01	-0.43	0.46
3	8	15	5	91.28	90.92	0.36	0.39
4	6	20	5	90.89	90.75	0.14	0.15
5	7	20	5	94.43	94.29	0.14	0.14
6	8	20	5	90.62	91.62	-1.00	1.10
7	6	25	5	91.28	91.67	-0.39	0.42
8	7	25	5	93.42	93.62	-0.20	0.21
9	8	25	5	90.33	89.36	0.97	1.07
10	6	15	6	87.46	87.95	-0.49	0.56



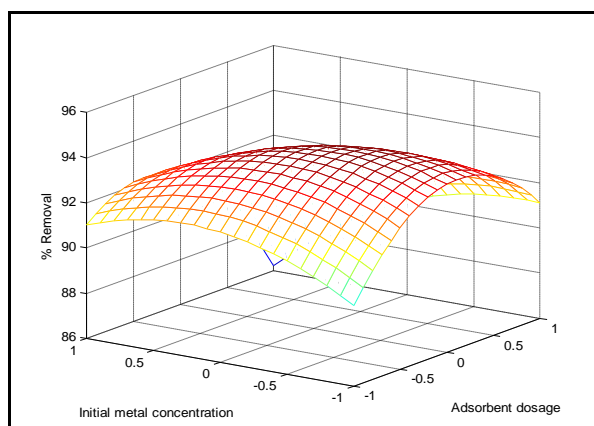
11	7	15	6	92.12	92.45	-0.33	0.35
12	8	15	6	91.11	90.74	0.37	0.40
13	6	20	6	91.02	90.99	0.026	0.02
14	7	20	6	94.82	93.91	0.91	0.95
15	8	20	6	89.78	90.61	-0.83	0.92
16	6	25	6	91.31	91.09	0.22	0.24
17	7	25	6	91.78	92.42	-0.64	0.69
18	8	25	6	87.41	87.53	-0.12	0.13
19	6	15	7	89.78	89.53	0.25	0.27
20	7	15	7	93.42	93.41	0.01	0.01
21	8	15	7	90.94	91.08	-0.14	0.15
22	6	20	7	91.18	91.75	-0.57	0.62
23	7	20	7	93.74	94.05	-0.31	0.33
24	8	20	7	90.71	90.12	0.59	0.65
25	6	25	7	91.44	91.03	0.41	0.44
26	7	25	7	91.69	91.74	-0.046	0.04
27	8	25	7	86.03	86.22	-0.19	0.22
28	7	20	6	94.82	93.91	0.91	0.95

The results of the regression model Eq. (11), in the form of ANOVA are presented in Table 4. Statistical testing of the model by the Fischer's statistical test for analysis of variance (ANOVA) yielded a F-value of 35.3 with a low p-value ( $P < 0.0001$ ) indicating the significance of the model. Determination coefficient nearer to unity ( $R^2 = 0.9464$ ) inferred that the quadratic model is the best fit for the observed adsorption of copper by DNF - CH composite. The predicted  $R^2$  of 0.8685 is in reasonable agreement with the adjusted  $R^2$  of 0.9196 (difference is less than 0.2).

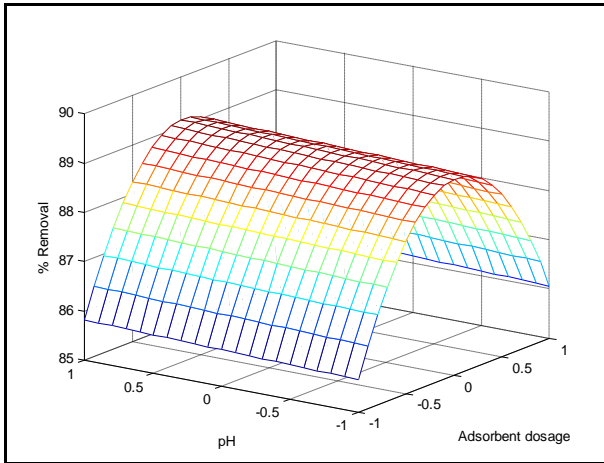
**Table 4. Regression coefficients for adsorption of copper by DNF-CH composite**

Model term	Coefficient estimate	Standard Error	F-Value	P-Value
Intercept	93.91	1.06	35.30	<0.0001
$X_1$	-0.19	0.55	1.68	0.2111
$X_2$	-0.016	0.55	0.011	0.9169
$X_3$	-0.12	0.55	0.68	0.4206
$X_1 X_2$	-1.59	0.68	77.98	<0.0001
$X_1 X_3$	-0.62	0.68	12.06	0.0027
$X_2 X_3$	-0.82	0.68	20.89	0.0002
$X_1^2$	-3.11	0.93	158.56	<0.0001
$X_2^2$	-1.47	0.93	35.64	<0.0001
$X_3^2$	0.26	0.93	1.07	0.3138

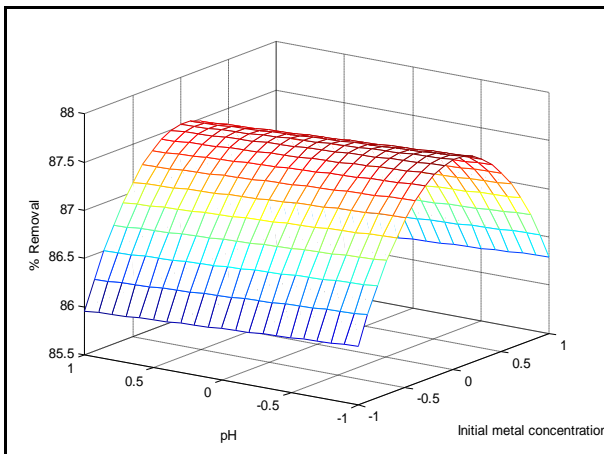
**Note:** All the linear, interaction and squared terms are significant ( $P < 0.5$ ), excluding linear  $X_2$ .



**Figure 9a. Surface plot for the effects of adsorbent dosage and initial metal concentration of copper on % removal**



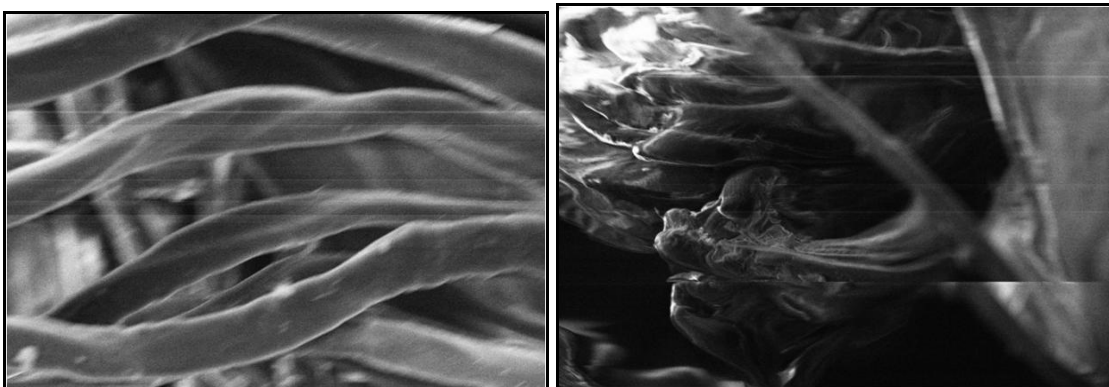
**Figure 9b.** Surface plot for the effects of adsorbent dosage and pH of copper on % removal



**Figure 9c.** Surface plot for the effects of pH and initial metal concentration of copper on % removal

### SEM - EDS analysis

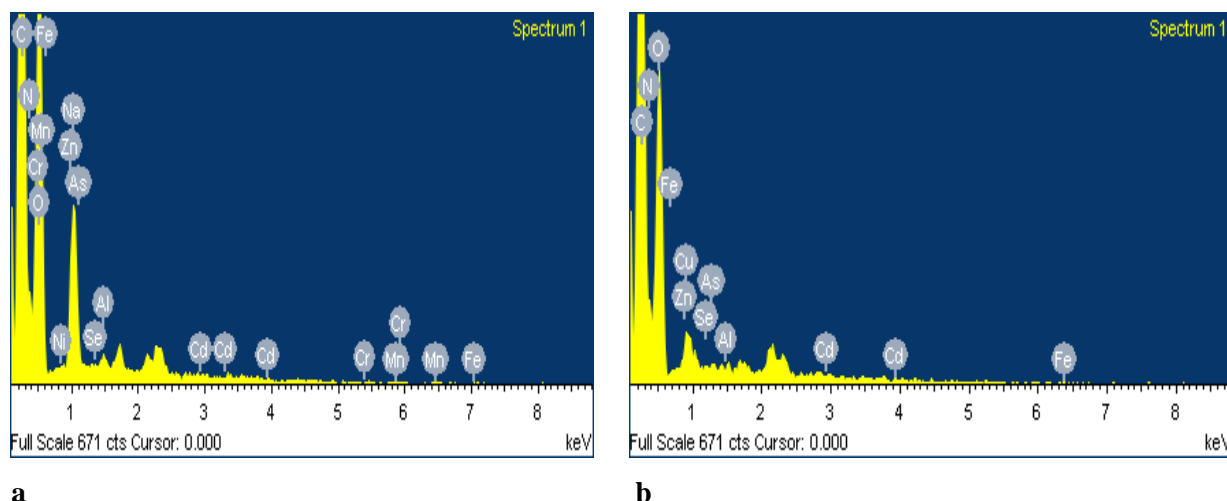
SEM analyses of the biosorbent material, DNF - CH composite, before and after adsorption of copper are presented in Figure 10. Before adsorption, the feather morphology was clear with barbs and barbules while after adsorption of copper, the distinction between the barbs and barbules is lost and the shrinkage is obvious. The major constituent of feather is keratin protein, along with N (chitosan provides amino groups), and metals Na, Mn, Zn, Fe, Al, Ni, Cd, As, Se, Cr etc. as is evident to be present in the composite from EDS analysis before biosorption (Figure 11a). After adsorption of copper (Figure 11b), the EDS analysis showed the presence of copper ions with a concomitant decrease in the percentage of As, Se and Cd and absence of Cr, Mn, Na, suggesting that the adsorption of copper by the composite may also include the ion-exchange mechanism.



**a**

**b**

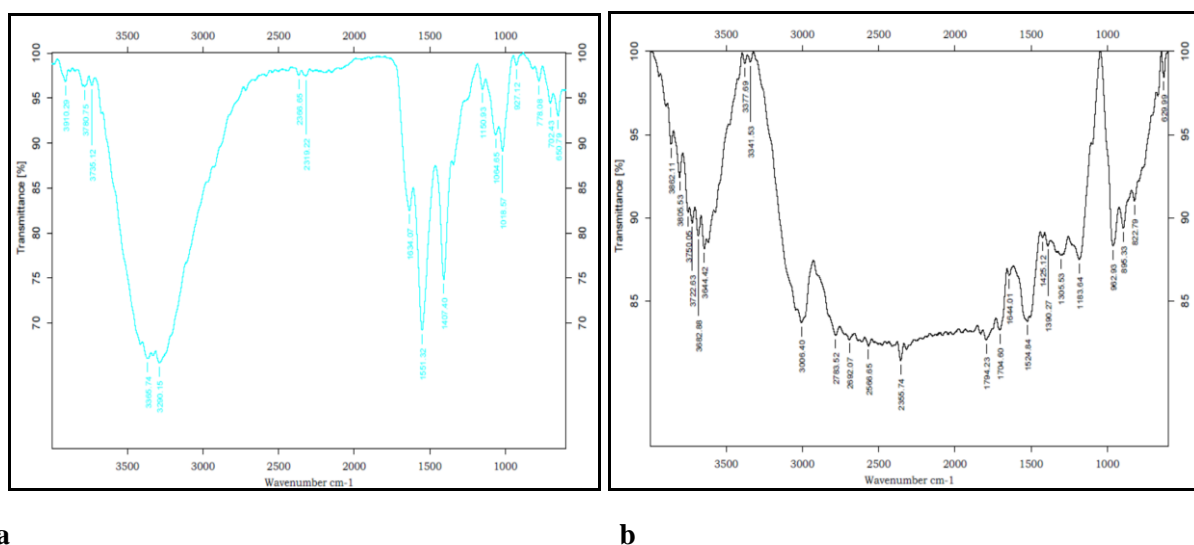
**Figure 10** SEM micrograph of DNF-CH composite a. Native b. After adsorption of copper



**a** **b**  
**Figure 11. EDS micrograph of DNF-CH composite a. Native b. after adsorption of copper**

### Adsorption mechanism through FTIR spectral analysis

The adsorption mechanism was investigated using FTIR analysis of native and metal loaded DNF-CH composite in the range of  $400\text{-}4000\text{cm}^{-1}$  (Figures 12a and 12b). In DNF-CH composite, Feather provided an efficient and strong support for the CH in forming the composite with more number of hydroxyl, carboxyl, amino and alcohol groups available to chelate the copper metal ions. FTIR spectra showed marked differences before and after adsorption of copper. Broadly, the spectrum could be divided into 3 distinct regions viz.  $630\text{-}1000\text{cm}^{-1}$ ,  $1180\text{--}3000\text{ cm}^{-1}$ , and  $3300\text{--}3700\text{ cm}^{-1}$  wherein there are significant variations in the absorption patterns and the groups assigned for biosorption.



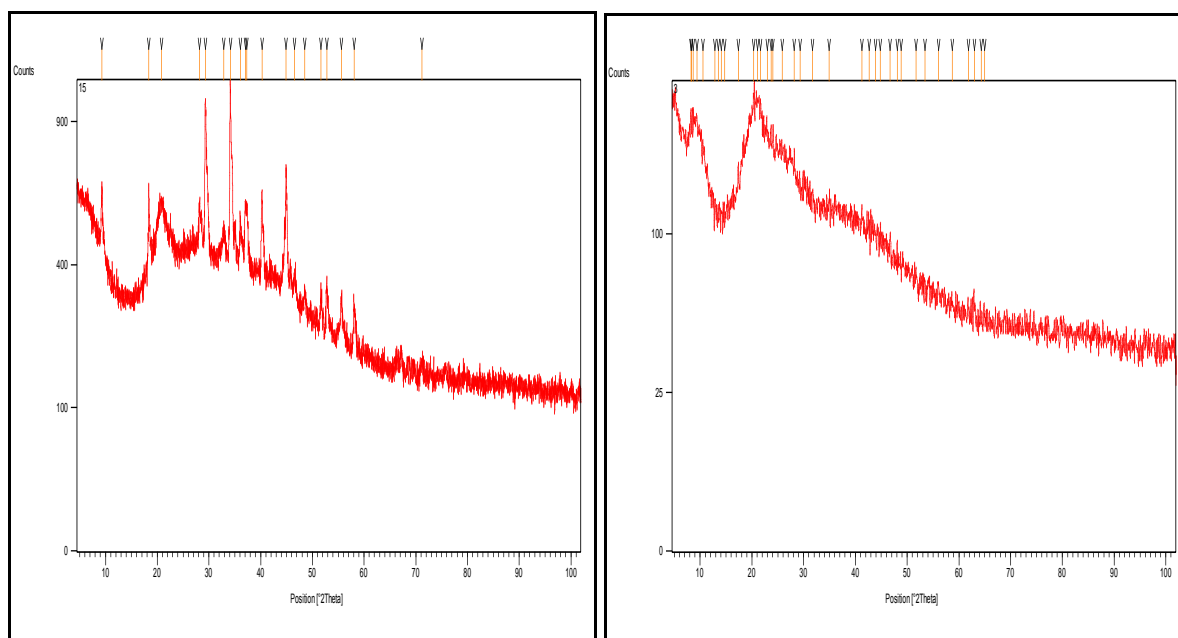
**a** **b**  
**Figure 12 FTIR spectra of DNF-CH Composite a. Native b. after adsorption of copper**

After interaction with the copper ions, it was identified that the sharp band at  $3735\text{ cm}^{-1}$  split in to  $3750\text{ and }3722\text{ cm}^{-1}$  indicating that both O and N atoms present on the adsorbent surface played a significant role in binding metal ions; the bands at  $3365\text{ cm}^{-1}$  were assigned to O-H & N-H; inter hydrogen bonds' vibration shifted to  $3377\text{ and }3341\text{ cm}^{-1}$ . C=O stretching in carboxyl or amide group shifted from  $1634\text{ to }1644\text{ cm}^{-1}$  and the intensity of the peak decreased. Moreover, the adsorption intensity for N-H bending vibration at  $1551\text{ cm}^{-1}$  shifted to  $1524\text{ cm}^{-1}$  and decreased after copper uptake. This observation is supported by an extreme change in absorption intensity for C-N stretching vibrations at  $1407\text{ to }1425\text{ cm}^{-1}$ . The metal ion uptake has led to a significant change in the absorption intensity of  $\text{NH}_3$  rocking vibration from  $778\text{ to }822\text{ cm}^{-1}$ . After exposure to copper, the presence of extra peaks at  $3682\text{ cm}^{-1}$ ,  $3644\text{ cm}^{-1}$ ,  $2783\text{ cm}^{-1}$ ,  $2692\text{ cm}^{-1}$ ,  $2566\text{ cm}^{-1}$  (O-H & N-H groups),  $1794, 1704\text{ cm}^{-1}$  (C=O stretching),  $1390\text{ cm}^{-1}$ ,  $1305\text{ cm}^{-1}$  (C-N stretching, in plane O-H bending) indicate the adsorption of copper. Variations in the absorption peaks at  $630\text{-}700\text{ cm}^{-1}$  in the two samples (before and after treatment with copper) are indicative of Cu-O stretching<sup>43</sup> in the copper loaded DNF-CH. In general, the adsorption capacity depends upon the porosity as well as functional groups present on the adsorbent surface<sup>44</sup>. Accordingly, the observed changes in adsorption intensity and the wave number of functional groups

strongly suggest the occurrence of complexation between N and O atoms on the adsorbent binding sites and the copper ions. The FTIR analysis suggests that the maximum adsorption of copper by the DNF-CH composite is due to the availability of more number of N and O atoms.

### X-ray diffraction (XRD) analysis

The DNF - CH X-ray diffractogram (as shown in Figure 13a) exhibited crystalline nature and showed sharp peaks at  $2\theta$  positions  $9^\circ$ ,  $18^\circ$ ,  $20^\circ$ ,  $28^\circ$ ,  $29^\circ$ ,  $32^\circ$ ,  $34^\circ$ ,  $36^\circ$ ,  $37^\circ$ ,  $40^\circ$ ,  $44^\circ$ ,  $46^\circ$ ,  $48^\circ$ ,  $51^\circ$ ,  $52^\circ$ ,  $55^\circ$ ,  $58^\circ$ , and  $71^\circ$ . Conversely, copper treated DNF-CH composite X-ray diffractogram analysis (as shown in Figure 13b) showed amorphous nature of the composite, with peaks at  $2\theta$  positions of  $9^\circ$ ,  $21^\circ$ ,  $23.6^\circ$ ,  $28^\circ$ ,  $34^\circ$ ,  $41^\circ$  and  $44^\circ$ . In addition, the peaks at  $43^\circ$  and  $50^\circ$  (JCPDS copper: 04-0836) indicate the presence of copper while the peaks at  $2\theta$  positions  $31.7^\circ$ ,  $34.9^\circ$  and  $46.7^\circ$  (JCPDS CuO: 80-1916) indicate the formation of CuO with hydroxyl and carboxyl groups present on the DNF. The presence of sharp peaks in the native adsorbent while their absence/reduction in copper treated adsorbent, as evidenced by SEM analysis, are suggestive of the adsorption of copper. This could be due to the electron pair sharing between copper metal and the carboxyl/hydroxyl groups in DNF-CH composite.



**a** **b**  
**Figure 13. XRD of DNF-CH composite a. Native b. After adsorption of copper**

### Conclusions

Equilibrium was attained at 70min of contact time due to the presence of more and/or active functional groups. The % removal of copper decreased with increase in the initial concentration of the adsorbate. The removal of copper increased rapidly with increasing adsorbent dosage initially and attained equilibrium at 7 g/L. Percentage removal of copper from aqueous solution increased significantly with increase in pH from 4 to 6; thereafter, increase in pH decreased the percentage removal. From Langmuir isotherm model, the maximum uptake capacity of 39.06 mg of copper per gram of the adsorbent was obtained at a temperature of  $30^\circ\text{C}$ . Maximum percentage of copper adsorption was found to be 93.91% (18.78 mg/L) and the residual concentrations of the metal after sorption corresponded to 1.22 mg/L, which is below the permissible limits (1.3 mg/L) of copper in drinking water. In a nut shell, the results of the present study demonstrate that DNF-CH composite could be considered for the safe, effective and economic treatment of the industrial waste water containing copper and achieve levels at par with the permissible limit of 1.3 mg/L of copper suggested for safe drinking water.

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## References

1. Sengil IA and Ozacar M. Biosorption of Cu(II) from aqueous solutions by mimosa tannin gel, J. Hazard. Mater., 2008, 157(2-3): 277-285.
2. Johnson PD, Watson MA, Brown J, Jefcoat IA. Peanut hull pellets as a single use sorbent for the capture of Cu(II) from waste water. Waste Manag., 2002, 22(5): 471-480.
3. Zhu CS, Wang LP, Chen WB. Removal of Cu(II) from aqueous solution by agricultural by – product: peanut hull. J. Hazard. Mater., 2009, 168(2-3): 739 - 746
4. Arul Manikandan N, Pakshirajan K and Syiem MB. Cu (II) removal by biosorption using chemically modified biomass of *Nostoc muscorum* - a cyanobacterium isolated from a coal mining site. Int. J. Chem. Tech. Res., 2014, 7(1): 80-92.
5. Batool R, Komal QA and Naeem A. Comparative study of Cr(VI) removal by *Exiguobacterium* sp. In free and immobilised forms. Biorem. J., 2014, 18(4): 317-327
6. Huang S. and Lin G. Biosorption of Hg(II) and Cu(II) by biomass of dried *Sargassum fusiforme* in aquatic solution. J Environ Health Sci Eng., 2015, 13: 21.
7. Kan SH, Sun BY, Xu F, Song QX and Zhang SF. Biosorption of aquatic copper (II) by mushroom biomass *Pleurotus eryngii*: Kinetic and isotherm studies. Water. Sci. Technol., 2015, 71(2): 283-288.
8. Wang JS, Hu XJ, Liu YG, Xie SB and Bao ZL. Biosorption of uranium (VI) by immobilized *Aspergillus fumigatus* beads. J. Environ. Radioact., 2010, 101(6): 504-508.
9. Ajjabai LC and Chouba L. Biosorption of Cu<sup>2+</sup> and Zn<sup>2+</sup> from aqueous solutions by dried marine green macroalga *Chaetomorpha linum*. J. Environm. Manag., 2009, 90(11): 3485-3489.
10. Amer MW, Ahmad RA and Awwad AM. Biosorption of Cu(II), Ni(II), Zn(II) and Pb (II) ions from aqueous solution by *Sophora japonica* pods powder. Int. J. Ind. Chem., 2015, 6(1): 67-75.
11. Putra WP, Kamari A, Yusoff SNM, Ishak CF, Mohamed A, Hashim N and Isa Imd. Biosorption of Cu(II), Pb(II) and Zn(II) ions from Aqueous solutions using Selected Waste Materials: Adsorption and Characterisation Studies. Journal of Encapsulation and Adsorption Sciences, 2014, 4(1): 25-35.
12. Gulbahar A. and Guzel F. Bioremoval and Recovery of Cu(II) and Pb(II) from Aqueous Solution by a Novel Biosorbent Watermelon (*Citrullus lanatus*) Seed Hulls: Kinetic Study, Equilibrium Isotherm, SEM and FTIR Analysis. Desalin. Water. Treat., 2013, 51(37-39): 7311-7322.
13. Shukla PM and Shukla SR. Biosorption of Cu(II), Pb(II), Ni(II), and Fe(II) on Alkali Treated Coir Fibers. Separation Science and Technology, 2013, 48(3): 421-428.
14. Hassan SB and Aigbodion VS. Effects of eggshell on the microstructures and properties of Al-Cu-Mg/eggshell particulate composites. Journal of King Saud University - Engineering Sciences, 2015, 27(1): 49-56.
15. Ratna Kumari A and Sobha K. Biosorption of Cu<sup>2+</sup> using animal polymers: Chick and duck feathers. International journal of research in pharmaceutical and biomedical sciences. 2012, 3(2): 664-669.
16. Yang C, Guan L, Zhao Y and Yan Y. Sorption of Cu<sup>2+</sup> and Zn<sup>2+</sup> by natural biomaterial: Duck feather. Appl. Biochem. Biotechnol., 2007, 142(2): 168-178.
17. Sun P, Liu ZT and Liu ZW. Chemically modified chicken feather as sorbent for removing toxic chromium(VI) ions. Industrial and engineering chemistry research, 2009, 48(14): 6882- 6889.
18. Venkatesa Prabhu S, Harish S, Narasimman S and Vignesh Chandra Y. Biosorption studies of heavy metals from synthetic effluents. International Journal of Bioprocess Technology, 2012, 1(1): 1-9.
19. Hossain A and Aditya G. Biosorption of cadmium from aqueous solution by shell dust of the Fresh Water Snail *Melanoides tuberculata*. Biorem. J., 2015, 19(1): 80-91.
20. Mohanasrinivasan V, Mishra M, Paliwal JS, Singh SKr, Selvarajan E, Suganthi V and Subathra Devi C. Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste. 3 Biotech, 2014, 4(2): 167-175.
21. Huang R, Yang B, Liu Q and Liu Y, Simultaneous adsorption of aniline and Cr(VI) ion by activated carbon/chitosan composite. J. Appl. Polym. Sci., 2014, 131(4): 39903-39912.
22. Shen C, Wang Y, Xu J and Luo G. Chitosan supported on porous glass beads as a new green adsorbent for heavy metal recovery. Chemical Engineering Journal, 2013, 229: 217- 224.
23. Haridi SSH, Zekry AA, Radwan MA and Sharaf MA. Evaluation and Characterization of Magnetic Carboxymethyl Chitosan Nanocomposite for Removal of Zink and Lead from Waste-water. Journal of American Science, 2012, 8(11): 56-60.
24. Guijuan JI, Weiwei BAO, Guimei GAO, Baichao AN, Haifeng ZOU and Shucai GAN. Removal of Cu (II) from aqueous solution using a novel crosslinked Alumina-Chitosan Hybrid Adsorbent. Chin. J. Che. Eng., 2012, 20(4): 641-648.

25. Zhang S, Zhou Y, Nie W and Song L. Preparation of Fe<sub>3</sub>O<sub>4</sub>/chitosan/ poly (acrylic acid) composite particles and its application in adsorbing copper ion (II). *Cellulose*, 2012, 19(6): 2081-2091.
26. Ramya R, Sankar P, Anbalagan S and Sudha PN. Adsorption of Cu(II) and Ni(II) ions from metal solution using crosslinked Chitosan –g- acrylonitrile copolymer. *International journal of environmental sciences*, 2011, 1(6): 1323-1338.
27. Rajiv Gandhi M, Kousalya GN and Meenakshi S. Removal of copper (II) using chitin / chitosan nano-hydroxyapatite composite. *Int. J. Biol. Macromol.*, 2011, 48(1): 119-124.
28. Tran CD, Mututuvvari TM. Cellulose, chitosan and keratin composite materials. Controlled drug release. *Langmuir*, 2015, 31(4): 1516-26.
29. Flores- Hernandez CG, Colin- cruz A, Velasco-santos C, Castano VM, Rivera-Armenta JL, Almendarez-Camarillo A, Garcia-Casillas PE, Martinez-Hernandez AL. All green composites from fully renewable biopolymers: chitosan-starch reinforced with keratin from feathers. *Polymers*, 2014, 6: 686-705.
30. Tanabe T, Okitsu N, Tachibana A, Yamauchi K. Preparation and characterization of keratin-chitosan composite film. *Biomaterials*, 2002, 23(3); 817-825.
31. Tachibana A, Furuta Y, Takeshima H, Tanabe T, Yamauchi K. Fabrication of wool keratin sponge scaffolds for long-term cell cultivation. *Journal of Biotechnology*, 2002, 93(2): 165-170.
32. Yu P. Molecular chemistry imaging to reveal structural features of various plant feed tissues. *J Struct. Boil.* 2005, 150(1), 81-89.
33. Ghaedi M, Mazaheri H, Khodadoust S, Hajati S and Purkait MK. Application of central composite design for simultaneous removal of methylene blue and Pb<sup>2+</sup> ions by walnut wood activated carbon. *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, 2015, 135: 479-490.
34. Srivastava NK, Parhi SS, Jha MK and Sreekrishnan TR. Optimization of effect of pre-treatment on Chromium removal by algal biomass using Response Surface Methodology. *International Journal of Engineering Research*, 2014, 3(3): 167-171.
35. Rafatullah M, Sulaiman O, Hashim R and Ahmad A. Adsorption of Copper(II) onto Different Adsorbents. *J. Dispersion. Sci. Technol.*, 2010, 31(7), 918-930.
36. Chen G, Fan J, Liu R, Zeng G, Chen A and Zou Z. Removal of Cd(II), Cu(II) and Zn(II) from Aqueous solutions by Live *Phanerochaete Chrysosporium*. *Environ. Technol.*, 2012, 33(22-24): 2653-2659.
37. Malkoc E. Ni(II) removal form aqueous solutions using cone biomass of *Thuja orientalis*. *J. Hazard. Mater.*, 2006, 137(2): 899-908.
38. Witek-Krowiak A, Szafran RG and Modelski S. Biosorption of heavy metals from aqueous solutions onto peanut shell as a low-cost biosorbent, *Desalination*, 2011, 265(1): 126-134.
39. Moreno-Pirajan JC, Garcia-Cuello VS and Giraldo L. The removal and kinetic study of Mn, Fe, Ni and Cu ions from wastewater onto activated carbon from coconut shells. *Adsorption*, 2011, 17(3): 505-514.
40. Tuzen M, Saygi KO, Usta C and Soylak M. *Pseudomonas aeruginosa* immobilized multiwalled carbon nanotubes as biosorbent for heavy metal ions. *Bioresource Technology*, 2008, 99(6): 1563-1570.
41. Grimm A, Zanzi R, Bjornbom E and Cukierman AL. Comparison of different types of biomasses for copper biosorption, *Bioresource Technology*, 2008, 99(7): 2559-2565.
42. Wang X, Li ZZ and Sun C. A comparative study of removal of Cu(II) from aqueous solutions by locally low-cost materials: marine macroalgae and agricultural by product. *Desalination*, 2009, 235(1-3): 146-159.
43. Vinod VTP and Miroslav C. Green synthesis of copper oxide nanoparticles using gumkaraya as a biotemplate and their antibacterial application, *Int. J. Nanomedicine.*, 2013, 8: 889-898.
44. Kumar PS, Ramalingam S, Senthamarai C, Niranjanaa M, Vijayalakshmi P and Sivanesan S. Adsorption of dyes from aqueous solution by cashew nut shell: Studies on equilibrium isotherm, kinetics and thermodynamics of interactions. *Desalination*, 2010, 261(1-2): 52 -60.

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