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Cu(II) removal by biosorption using chemically modified biomass of *Nostoc muscorum* – a cyanobacterium isolated from a coal mining site

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Abstract: This studyinvestigated Cu(II) removal by biosorption using acid treated *Nostoc muscorum* (AT-NM) – a cyanobacterium, its detailed characterization and optimization employing Box Behnken design (BBD) under Response Surface Methodology (RSM). The process parameters and their optimized levels were: 5.24 initial solution pH, 17.33 mg/L initial metal concentration, 1.1 g/L biosorbent dosage and 3hequilibrium time. At these optimized conditions, a maximum Cu(II) removal of 89%was achieved, which was 3% higher than value obtained under unoptimized conditions. Characterization of the biosorbent using Fourier transform infrared (FTIR) spectroscopyrevealed the presence of N-H, C-H, CH=CH, CH_2, C-O-C functional groups in AT-NM for Cu(II) biosorption. A high BET surface area of 0.996 m²/g of the biosorbent further revealed its very high suitability for Cu(II) biosorption. Cu(II) sorption kinetics by AT-NM followed the pseudo second-order kinetics with an estimated coefficient of determination (R^2) value of 0.999. The Weber and Morris kinetic model fitting of the data confirmed that Cu(II) removal by AT-NM is due to its surface binding. Among the different isotherm models tested in the study, Freundlich isotherm was found to accurately fit the experimental data with a maximum R^2 value of 0.993. All these results revealed an excellent potential of AT-NM for heavy metal removal from wastewater.
Keywords: Biosorption, *Nostoc muscorum*, Cu(II) removal, Box Behnkhen Design, Response Surface Methodology.

1. Introduction

Treatment of metal bearing industrial wastewater is of heightened interest[1,2]. This is mainly because heavy metals are toxic even at a very low concentration to human, animal and plant species. For instance, copper at a concentration higher than 1 mg/L can cause several liver related ailments in humans[3]. It is therefore essential to reduce concentration of heavy metals like Cu(II) below its permissible limit from wastewater prior to its discharge into the environment.

Amongst the different known techniques to remove heavy metals from wastewater, biosorption seems to be cost effective as it involves only a passive process for heavy metal sequestration by using mostly dead/inactive and cheap biomass[4]. Also, among the different biomass types available for heavy metal removal, the cyanobacterial biomass is particularly attractive owing to its complex biomass structure, minimum nutrient requirement, abundant growth within a short time period, ability to grow well under environmentally stressed conditions etc. The use of dead cyanobacterialbiomass is also advantageous because it doesn't require any kind of special media for its growth[5]. Further, it has been reported that chemical pretreatment of such biosorbents show a large ability to form complex with metal ions, thus aiding in their efficient removal from aqueous solution[6].

As mentioned in Table 1, there are only a few reports on Cu(II)biosorptionusing rawalgal species, and there are no reports on chemically modified biomass of cyanobacteria such as *Nostoc muscorum,* whichhas otherwise beenreported in the literature for their abundant growth within a short span of time even under environmentally stressed conditions[3]. Hence, the present study was aimed at studying Cu(II) biosorption using acid treated biomass of *N. muscorum* (AT-NM) as a low cost biosorbent. The cyanobacterium used in this study was initially isolated from a coal mining contaminated site and the live biomass tested to remove heavy metals from aqueous solution using bioaccumulation process.

Table 1Literature reports on Cu(II) removal process conditions.

S. no.	Biosorbent	Process parameters				Copper removal (%)	References
		initial metal concentration (mg/L)	Initial solution pH	Temp. (^oC)	Biosorbent dosage (g/L)		
1	*Ulvafasciata sp*	100	5	30	0.1	26.88	[7]
2	*Chlorella Vulgaris sp*	100	4.5	25	1	60	[8]
3	*Chlorella Vulgaris*	100	4	25	0.75	37.6	[9]
4	*Spirulinaplantensis*	20	6	25	1	50	[10]
5	**Acidtreated*Nostoc muscorum***	**17.33**	**5.24**	**35**	**1.1**	**89**	**This study**

Prior to the Cu(II) biosorption experiments, detailed characterization of the AT-NM using Fourier Transform Infrared (FTIR) spectroscopy, Field Emission Scanning Electron Microscopy (FESEM) and Brunauer Ernmet Teller (BET) surface area analysis was carried out. Besides, the effect of various process parameters, viz. pH, initial metal concentration and AT-NM dosage, on Cu(II) biosorption by AT-NM was examined and their levels optimized using Box Behnken Design (BBD) of experiments under Response Surface Methodology (RSM). BBD-RSM was used in the optimization study in order to minimize the number of experimental runs as well as to procure maximum information with regard to the individual and interaction effects of these parameters affecting the Cu(II) biosorption[11,12,13].

2. Materials and methods

2.1. *N. muscorum* growth and culture conditions

The cyanobacterium*Nostoc muscorum* was initially isolated from a coal mining site in Meghalaya, India [11] and it was routinely cultured using Blue Green (BG-11) media [14]. Composition of the BG -11 media (per litre) was: citric acid: 0.006 g, ferric citrate: 0.006 g, EDTA (disodium salt): 0.001 g, Na_2CO_3: 0.02 g, $MgSO_4 \cdot 7H_2O$: 0.075 g, $CaCl_2 \cdot 2H_2O$: 0.036 g, K_2HPO_4: 0.04 g, $MnCl_2 \cdot 4H_2O$: 1.81 mg, Na_2MoO_4: 0.039 mg, H_3BO_3: 2.86 mg, $CuSO_4 \cdot 5H_2O$: 0.079 mg, $Co(SO_4) \cdot 7H_2O$: 0.04 mg and $ZnSO_4 \cdot 7H_2O$: 0.222 mg. The cyanobacterium was grown on BG-11 media with a fluorescent light illumination of 50 µmol/m^2s following conventional 12 hr light and 12 hr dark cycles at a temperature of 28^oC [15].

2.2. Biosorbent preparation

For Cu(II) biosorption experiments, 15 day old *N. muscorum* culture was centrifuged and the obtained biomass was washed with distilled water to remove any surface adhering media components. The biomass was then dried overnight at 65^oC and ground using a mortar and pestle. The ground biomass was treated with 0.5M HCl for 5 min followed by washing with distilled water. The acid treated *N. muscorum* (AT-NM) was subsequently used as the biosorbent in all Cu(II) biosorption experiments in this study.

2.3. Biosorbent characterization

Analysis of AT-NM surface area, pore volume and pore diameter was carried out using Brunauer Emmet Teller (BET) equation based automated adsorption apparatus (SA 3100,BeckhenCoulter, Switzerland) with nitrogen as the adsorbate[16].

Functional groups present in untreated *N. muscorum* biomass, AT-NM and Cu(II) loaded AT-NM were analyzed using a FTIR spectroscope (IR Affinity, Shimadzu, USA). For this FTIR analysis,1mg each of the different biomass samples was mixed with 100 mg of dried KBr powder and pressed to a sheer slice. An average of 5 scans was collected for each measurement in the range of 400-4000 cm^{-1} wave number with a resolution of 4 cm^{-1}[17].

FESEM images of untreated *N. muscorum* biomass, AT-NM and Cu(II) loaded AT-NM were acquired using a scanning electron microscope (Sigma, Zeiss, Switzerland). The sample images were recorded at operating conditions of 3.0 kV and 200 nm[18].

2.4. Cu(II) biosorption experiments

For Cu(II) biosorption experiments using AT-NM, a 1000 mg/L stock solution of the metal was prepared by dissolving $CuSO_4 \cdot 5H_2O$ in deionized water and diluted as necessary.All biosorption experiments in this study were carried out in triplicate using 250 ml Erlenmeyer flasks with a working volume of 100 ml Cu(II) solution. Initial pH of the metal solution was adjusted using 0.1M HCl and 0.1M NaOH. The flasks were agitated on a rotary shaker set at 120 rpm speed and at 35^{o}C temperature. At the end of each batch experiment, samples were taken and centrifuged at 5000 ×g for 5 min. The biomass free supernatant obtained was analyzed for residual Cu(II) concentration using an atomic absorption spectrophotometer (AA 220, Varian, The Netherlands).

2.4.1Effect of process parameters on Cu(II) removal and its optimization

The effect of contact time on Cu(II) biosorption by AT-NM was first studied by determining the residual Cu(II) concentration at different time intervals for a total time period of 4 h. The other parameters that were held constant for this experiment were initial solution pH of 6, initial metal concentration of 10 mg/L, AT–NM dosage of 0.1 g. The metal removal in this batch experiment was expressed as uptake capacity (Q_e) as given by (Eq. 1):

$$Qe\left(\frac{mg}{g}\right) = \frac{v(Co - Ce)}{w} \qquad (1)$$

where,C_0 is the initial metal concentration (mg/L), C_e is the equilibrium or final metal concentration (mg/L); v is the volume of metal solution (L); and w is the dry weight of AT–NM (g).

In order to study the effect of other process parameters, namely initial metal concentration, initial solution pH and AT-NM dosage, on Cu(II) removal by AT-NM, experiments were carried out as per three factor three level BBD[3,19]. Combination of levels of these three parameters in each experimental run is presented in (Table 2), which shows that each parameter level was coded as either -1,0 or +1 for statistical analysis purposes. In case of initial Cu(II) concentration,these coded levelscorresponded to 10, 55 and 100 mg/L, respectively; whereas, in case of initial solution pH, these levels were 3, 6 and 9, respectively. For AT-NM dosage 1, 6.5 and 12 g/Lcorresponded to the three coded levels -1,0 or +1. All these levels of these parameters were chosen based on a literature study by Kiran[3] for Cu(II) biosorption. The results of Cu(II) removal in all these experimental runs was expressed as percentage as per the following (Eq.2).

$$Cu(II)\ Removal\ (\%) = \frac{Co - Ce}{Co} X100 \qquad (2)$$

where,$C_0$is initial metal concentration (mg/L) and C_e is equilibrium metal concentration (mg/L).

Table 2 Box-Behnken design matrix showing the process parameters and their levels along with the observed response (working volume: 100 ml, total sorption time: 3 h, solution temperature: 35°C)

Exp. run no.	Initial metal concentration (mg/L)	Initial solution pH	AT–NM dosage (g)	Cu (II) removal (%)
1	10.00	6.00	0.10	86
2	100.00	6.00	0.10	31.2
3	100.00	6.00	1.20	32.7
4	55.00	3.00	0.10	36.2
5	55.00	9.00	0.10	64.6
6	10.00	9.00	0.65	74.2
7	10.00	6.00	1.20	79.3
8	100.00	9.00	0.65	28.6
9	55.00	6.00	0.65	85.8
10	10.00	3.00	0.65	72.6
11	55.00	6.00	0.65	81.2
12	100.00	3.00	0.65	14.8
13	55.00	6.00	0.65	85.8
14	55.00	9.00	1.20	32.3
15	55.00	3.00	1.20	47.18

For statistical analysis of the results, the data was fitted to a second order polynomial equation of the general form shown in Eq.(3)[20,21].

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ii} x_j^2 + \sum b_{ij} x_i x_j \qquad (3)$$

where, x_i, x_j denote independent parameters, Y is % Cu(II) removed by biosorption, b_0 is the offset term, b_i is the first order main effect, b_{ii} is the second order main effect and b_{ij} is the interaction effect.

The second order polynomial equation, involving the actual parameters initial metal concentration, initial solution pH and AT-NM dosage, obtained using the statistical software Design Expert™ (version 7.1.5, Stat-Ease Inc., USA) is represented in Eq. (4).

$$Y = 47.20 + 11.66A + 2.3B + 1.26C + 1.89AB + 0.54AC - 1.06BC - 16.56A^2 - 11.63B^2 - 10.53C^2 \quad (4)$$

Where, Y is the Cu(II) removal, A-initial metal concentration, B-pH, C- AT-NM Dosage, A^2,B^2,C^2 are the quadratic effects of the respective parameters. The goodness of the fit of this quadratic equation was analyzed using the Analysis of variance (ANOVA).

2.4.2 Cu(II) sorption kinetics and isotherm

The kinetics of Cu(II) sorption by AT-NM was studied by fitting the experimental data on Cu(II) removal at different contact time to the Lagergren's pseudo first-order, Ho's pseudo second-order and Weber and Morris kinetic models. These models are briefly described further.The pseudo-first order rate expression is defined as in Eq.(5)[3].

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \qquad (5)$$

where, q_e and q_t, both expressed in mg/g, are biosorption capacities at equilibrium and at time t (min), respectively, and k_1(min^{-1}) is the pseudo first-order rate constant. The values of k_1 and $q_e$were calculated from the slope and intercept, respectively,of a linear plot of log (q_e- q_t) versus t.

The pseudo second-order kinetic model, which is based on chemisorption theory, is presented by Eq. (6). [22 and 23].

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (6)$$

where, k_2(g/mgmin) is the pseudo second-order rate constant[23]. The parameters q_e and $k_2$were obtained from the slope and intercept, respectively, of a linear plot of t/q_t versus t.

Weber and Morris kinetic model is based on intraparticle diffusion mechanism and is given by Eq.(7)[24].

$$q = f\left(\frac{Dt}{r_p^2}\right) = k_i t^{1/2} \qquad (7)$$

where, r_p (cm) is AT-NM radius, D (cm^2/min) is the solute diffusivity into the particle, q_t (mg/g) is the metal ions adsorbed at a specific time, and k_i is the intraparticle rate constant (mg/g min).A plot of q_t versus $t^{1/2}$should yield a straight line through the origin if intraparticle diffusion is the rate determining step.

For determining Cu(II) sorption isotherm parameters in this study, Cu(II) sorption experiments was carried out at different initial Cu(II) concentration of 10, 55 and 100 mg/Lwithan initial solution pH of 5.4 and AT-NM dosage of 1.1 g/L. The results obtained as q_e were fitted to Langmuir, Freundlich and Temkin isotherm models. These models are briefly discussed further:

The Langmuir isotherm model is based on monolayer sorption theory as expressed in Eq. (8)[25].

$$\frac{C_e}{q_e} = \frac{1}{k_L q_m} + \frac{C_e}{q_m} \qquad (8)$$

where, q_e (mg/g) is the equilibrium Cu(II) sorption capacity, C_e (mg/L) is the Cu(II) concentration in solution at equilibrium. The model parameters q_m (mg/g) and K_L (L/mg) relates to maximum sorption capacity and rate of Cu(II) sorption, which are obtained from slope and intercept, respectively, of a linear plot of C_e/q_e versus C_e.

The empirical Freundlich isotherm proposes a multilayer sorption with a heterogeneous energetic distribution of active sites, accompanied by interactions between adsorbed molecules and can be expressed as in Eq. (9)[26].

$$\log q_e = \log k_F + \frac{1}{n}\log C_e \qquad (9)$$

where, K_F (mg/g) and n (g/L) are the Freundlich constant and heterogeneity factor, respectively. Whereas K_F is related to bonding energy between Cu(II) and AT-NM; 1/n value is related to its sorption intensity with value ofn greater than 1 representing a favourable sorption process[26, 27].

The Temkin isotherm model is framed on the basis that heat of sorption of all the molecules in surface decreases linearly due to the coverage of the sorption sites. It further states that sorption takes place due to the energy in the bonding sites. The empirical Temkin expression can be written as in (Eq. 10)[3].

$$Q_e = 1/b_{tm} RT \ln K_{tm} + 1/b_{tm} RT \ln C_e \qquad (10)$$

where, T is the absolute temperature (K), R is the universal gas constant (8.314 J/mol/K), K_{tm} is the equilibrium binding constant (L/mg), and b_{tm} is the variation of sorption energy (kJ/mol).The goodness of the fit of these models was determined by their respective coefficient of determination (R^2) values.

3. Results

3.1. Biosorbent characterization

(Fig.1) shows the FT-IR spectra of raw/untreated *N.muscorum*, AT-NM and Cu(II) loaded AT-NM biomass, which clearly reveals changes in the peaks of raw *N. muscorum* biomass due to acid treatment as well as due to Cu(II) loading onto AT-NM. The different peaks in the spectra of untreated*N. muscorum* also reveal a complex nature of the biosorbent through the presence of different functional groups.Shift in these peaks were generally observed in the AT-NM and Cu(II) loaded AT-NM. In particular, a shift in the peak from 3443.15 cm^{-1}corresponding to N-H group on the raw *N. muscorum*to 3743.97 cm^{-1} following acid treatmentof the biomass[6, 12]. The peak was further shifted to 3438.44 cm^{-1} following Cu(II) biosorption onto AT-NM, thus confirming the involvement ofN-H group on AT-NM for copper loading. Inaddition, a change in the peak from 2925.50 cm^{-1} in raw biomass (before acid treatment) to 2923.87 cm^{-1} in AT-NM followed by a further change to 2854.14 cm^{-1} in Cu(II) loadedAT-NM strongly revealed the participation of C-H functional groups inthe biosorptionprocess (Fig.1and Table 3)[12, 13].

Fig.1 and Table 3also reveala shift in the peak at 1645.11 cm^{-1} in the raw biomass to 1653.30 cm^{-1} following acid treatment. Further shift in the peakto 1654.50 cm^{-1}due to copper biosorption onto AT-NMindicated the involvement of-CH=CH-group in the AT-NM for Cu(II) binding[4,13]. Furthermore, the spectra revealed the presence of alkyl and ester groups on the raw biomass owing to their corresponding peaks at 1460.96 cm^{-1} and 1202.24 cm^{-1}(Fig.1). Subsequent shift in these peaks was found in the spectra of Cu(II) loaded AT-NM confirming the involvement of these groups in the Cu(II) biosorption by AT-NM. All these results of FT-IR analysis, shift in peak due to acid treatment of *N. muscorum*and Cu(II) biosorption,and the corresponding functional groups are summarized in Table 3.

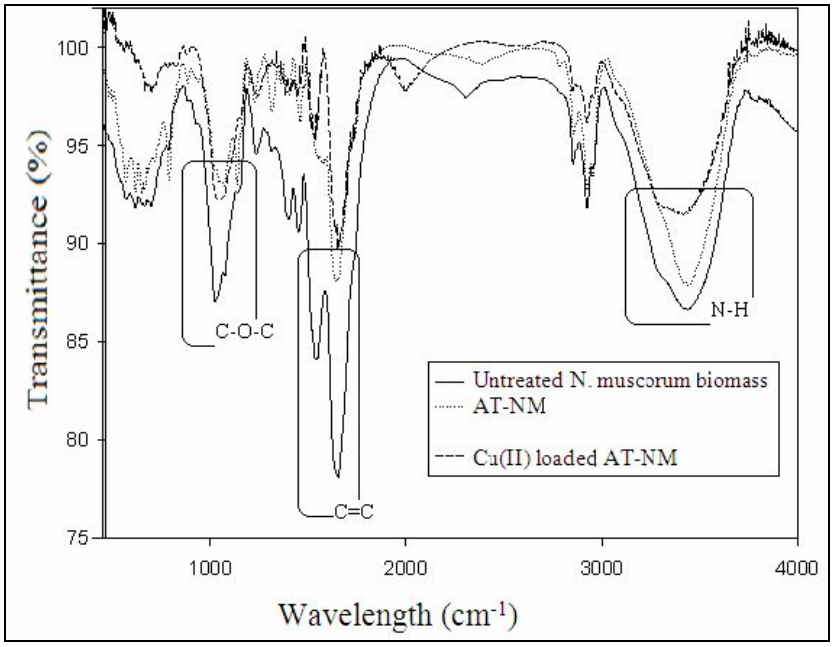


Fig. 1.FTIR spectra of raw *N. muscorum* biomass, AT-NM and Cu(II) loaded AT-NM.

Table 3 Results of FT-IR analysis of raw *N. muscorum*, AT-NM and copper loaded AT-NM revealing the presence of different functional groups involved in Cu(II) biosorption

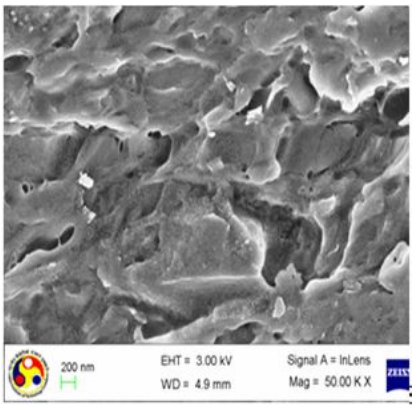
S. No	Presence of peak (cm^{-1})			Functional group corresponding to the peak	Compounds containing the functional group
	Before acid treatment	After acid treatment	After copper loading		
1	3443.15	3743.97	3438.44	N-H	Proteins
2	2925.50	2923.87	2854.14	C-H	Carbonyl compound
3	1645.11	1653.30	1654.50	CH=CH	alkenes
4	1460.96	1537.83	1544.52	CH_2	Alkanes
5	1202.24	1234.94	1235.44	C-O-C	Lipids

Table 4shows the BET surface area analysis of AT-NM biomass following thenitrogen adsorption method. This analysis revealeda surface area of 0.996 m^2/g, a pore volume of 0.006 ml/g and a pore diameter of 8.198 nm of the biosorbent. The value of BET surface area is also much higher compared to that of algal biosorbents reported in the literature (Table 4).

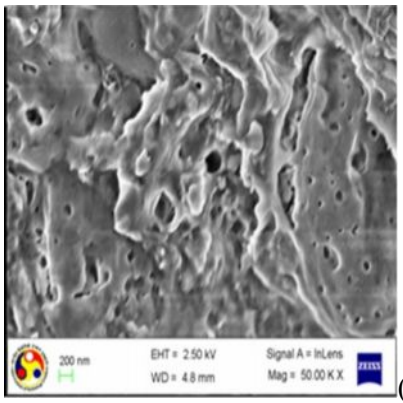
Table 4 AT-NMBET surface area in comparison with algal biosorbents reported in the literature

S.No	Biosorbents	Surface Area (m^2/g)	References
1	*Algae Gelidium*	0.23 ± 0.01 28	28
2	*Mucorheimalis*	0.2057	29
3	*Undariapinnatifida*	0.3	30
4	*Macrocystispyrifera*	0.2	30
5	*Fucusvesiculosus*	0.19± 0.01	31
6	Acid treated *Nostoc muscorum*	0.99± 0.01	This study

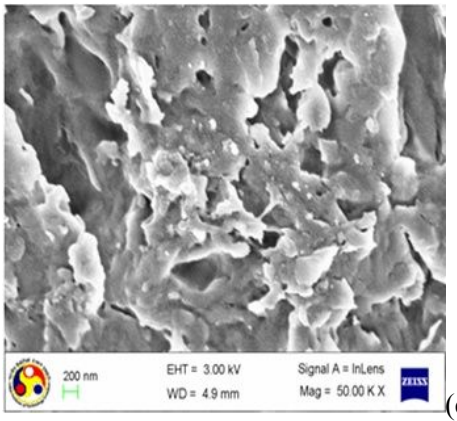
The FESEM analysis results (Fig.2) revealed the morphological changes in raw *N. muscorum* biomass due to acid treatment and Cu(II) biosorption. In particular, AT-NM was more porous as compared to raw biomass, and the AT-NM pores were diminished due to Cu(II) biosorption (Fig. 2c). These results strongly revealed Cu(II) binding to the pores of the AT-NM [12].



(a)



(b)



(c)

Fig. 2.FESEM image of (a) untreated *Nostoc muscorum* biomass, (b) AT-NM and (c) Cu(II) loaded AT-NM.

3.2. Effect of parameters on Cu(II) sorption and process optimization

Fig.3 shows the effect of contact time on Cu(II) biosorption by AT-NM, which reveals that a maximum sorption took place within the first fifteen minutes of contact itself. At a later time, the biosorption only slightly improved and eventually, at the end of 3h, itremained constant.

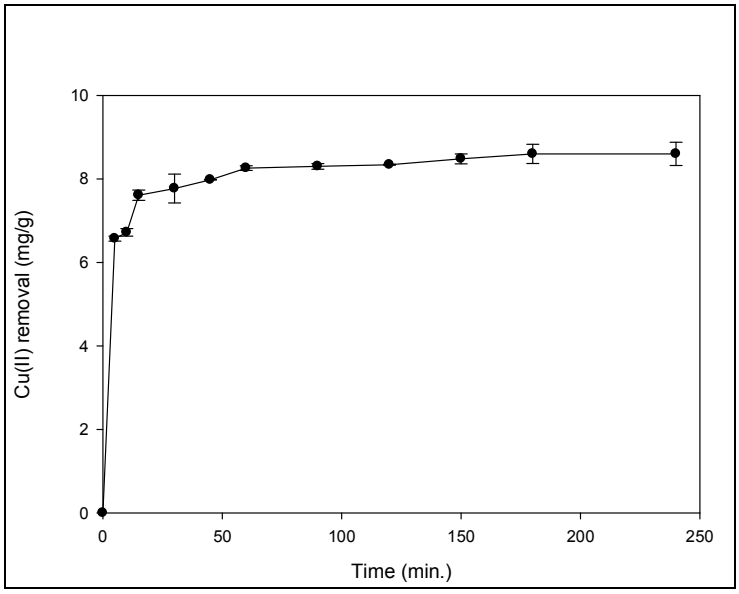


Fig. 3. Effect of contact time on Cu(II) removal by AT-NM (initial solution pH = 6, initial metal concentration = 10 mg/L, AT–NM dosage = 0.1 g, working volume = 100 ml, solution temperature = 35 ^{o}C)

To study the effect of the other parameters, experiments were carried out as per the BBD(Table 2),which shows a maximum Cu(II) removal of 86% at an experimental combination of initial solution pH of 6, initial metal concentration of 10 mg and biosorbent dosage of 1 g/L. The data also reveals variation in the response due to the different combination of the levels of the parameters, thus indicating a strong role of these parameters and their interactions on the Cu(II) removal by AT-NM[32,33]. In order to further determine which of these parameters and their interactions were significant on the Cu(II) biosorption process, analysis of variance (ANOVA) of the results were carried out based on the quadratic equation model depicted earlier in Eq. (4). Table 5 shows the ANOVA of Cu(II) removal by AT-NM which, in general, shows that a model parameter with a large F-value and a corresponding P-value is highly significant on Cu(II) removal [34,35]. Thus, all the three parameters (initial metal concentration, biosorbent dosage and initial solution pHwere highly significant on the Cu(II) biosorption process. Among these parameters, initial metal concentration proved to be the most significant with a P-value of <0.0001 followed by the equally significant AT-NM dosage and initial solution pH with P-values of 0.0287 and 0.021, respectively. In addition to these significant effect of theseindividual parameters, interaction effect between initial solution pH and biosorbent dosage was also found to be highly significant with a P-value of <0.0001. The "high P-value" for the lack of fit of the model (Table 5) together with a high value of R^2 (0.9908) further confirmed the validity of the quadratic equation model in description of these results of effect of parameters on the Cu(II) biosorption process[3].

Table 5 ANOVA of Cu(II) removal by AT–NM in the RSM study

Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**F**	**P- value**
Model	9114.51	9	1012.72	106.53	<0.0001
A-initial metal concentration	5242.88	1	5242.88	551.53	<0.0001
B-pH	104.55	1	104.55	11.00	0.0211
C-AT-NM biosorbent dosage	87.91	1	87.91	9.25	0.0287
AB	37.21	1	37.21	3.91	0.1048
AC	16.81	1	16.81	1.77	0.2410
BC	468.29	1	468.29	49.26	0.0009
A^2	553.47	1	553.47	58.22	0.0006
B^2	2211.49	1	2211.49	232.64	<0.0001
C^2	800.41	1	800.41	84.20	0.0003
Residual	47.53	5	9.51		
Lack of fit	33.42	3	11.14	1.58	0.4103

The quadratic equation (Eq.4) depicting the Cu(II) removal by AT-NM based onwhich the above mentioned ANOVA results were deduced, was further solved to determine the optimum level of the process parameters in enhancing the Cu(II) removal by *N.muscorum*. Thus, at an optimum condition of initial metal concentration 17.33 mg/L, initial solution pH 5.4 and AT-NM dosage 1.1 g/Lfor an equilibrium time of 3h,the Cu(II) removal was found to be 89% , which is 3% higher than the value obtained under unoptimized conditions (Table 2).

3.3. Cu(II) biosorption kinetics and isotherm

For understanding the Cu(II) sorption mechanism in this study, the data on Cu(II) biosorption by AT-NM at different time intervals and at different initial metal concentration, but keeping the other parameters at their respective optima,was fitted to suitable models found in the literature. These models were earlier described in the section 2.4.2.The results of kinetic model fitting are presented in Table 6, which reveals that only the pseudo second-order kinetic model fitted the Cu(II) biosorption kinetics actually with a R^2 value close to 1. The estimated model parameter q_e value of 0.115 g $mg^{-1}min^{-1}$ also matched well with the experimental q_e value. From the slope of a plot between q_e vs $t^{0.5}$ due to the Weber and Morris kinetic model, the k_p value was further estimated to be 0.164 mg/g min.Among the different Cu(II) isotherm models tested in this study, the freundlich model gave an accurate fit with aR^2 value of 0.993(Table 7). The other two models, Langmuir and Temkin, yielded only a slightly less accurate fit with R^2 values of 0.923 and 0.928,respectively. Further, the n-value estimated from the Freundlich model was high (1.66 g/L), thus indicating a favourable nature of the Cu(II) biosorption process.

Table 6 Estimated Cu(II) sorption kinetic parameters of pseudo first-order and pseudo second-order kinetic models

Parameter	Pseudo first–order model	Pseudo second-order model
q_e	6 (mg/g)	9 (mg/g)
k	0.183(min^{-1})	0.115(g/mg·min)
R^2	0.974	0.999

Table 7 Estimated Cu(II) sorption isotherm model parameters

Langmuir isotherm			Freundlich isotherm			Temkin isotherm		
q_m (mg/g)	K_L (L/mg)	R^2	n g/L	K_F (mg/g)	R^2	k_{Tm} (L/mg)	b_{Tm} (kJ/mol)	R^2
0.063	7.298	0.923	1.66	1.533	0.993	0.0070	6.196	0.928

4. Discussion

4.1. Cu(II) biosorption by AT-NM

Characterization of the different *N.muscorum*biomass samples (raw, AT-NM and Cu(II) loaded AT-NM)using FT-IR revealed a major involvement of the N-H group (Fig.1 and Table 4),probably present in the protein part of the biomass [6,12]. The FT-IR results alsorevealedthe involvement of CH=CH,which is a well-known functionalgrouppresent in lipid component of biomass. Therefore, it is likely that these charged functional groups were responsible for binding the positively charged Cu(II) ions. The Cu(II) sorption onto AT-NM was further elucidated by the results of kinetic and isotherm analyses (Tables 6 and 7). Whereas the accurate pseudo second-order kinetic model fitting of the data revealed intraparticle diffusion of Cu(II) ion as the rate controlling step, a better fit due to the Freundlich model indicated that the Cu(II) sorption follows multilayer isotherm theory [26]. Moreover, the small value of 1/n of the Freundlich model revealed a strong affinity and favourable sorption of Cu(II) on to AT-NM.In addition,from a plot of q_eVs $t^{0.5}$ according to the Weber and Morris kinetic model, two different lines were obtained mainly due to the immediate surface adsorption followed by the intra particle diffusion of Cu(II) ion. This further explains a maximum removal of

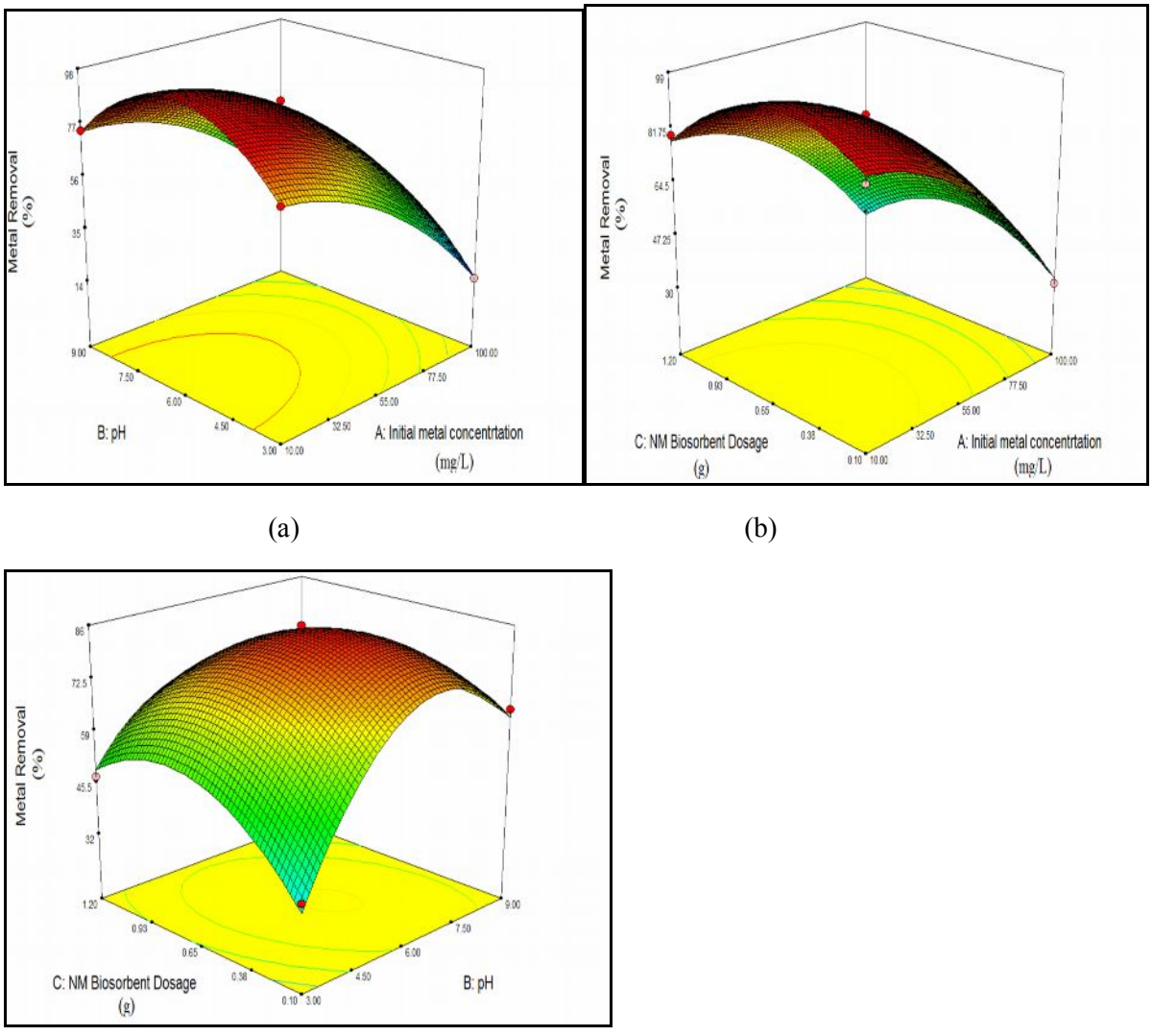
Cu(II) i.e., 76.1% within the first fifteen minutes of contact time due to its surface sorption onto the biosorbent. The later removal is attributed to the intraparticle diffusion of Cu(II) ions (Fig.3) [24,36].

Whereas FESEM image (Fig.2) of the different biomass showed a porosity decrease in AT-NM following Cu(II) biosorption, BET surface area of the biosorbent revealed a large value of 0.99 m^2/g (Table 4). Both these characterization result indicate a highly favourable nature of the biosorbent for Cu(II) removal from aqueous solution.

4.2. Effect of Cu(II) sorption parameters

The effect of contact time on Cu(II) biosorption indicated that the process is very rapid with an equilibrium achieved at the end of 3h itself (Fig 3). This can be attributed to the reduced surface mass transfer as a result of decrease in Cu(II) concentration gradient between the solution and the biosorbent with an increase in time[27]. This could also be due to the complete saturation of Cu(II) binding sites on AT-NM with time[37]. Among the other parameters (initial solution pH, initial metal concentration and AT-NM dosage), initial metal concentration was highly significant on Cu(II) removal (Tables 2 and 5). Moreover, it was observed that the Cu(II) removal decreases with an increase in the initial metal concentration,which is mainly due to availability of more metal than the available binding sites on the biomass. Hence a maximum of 86% removal was obtained for an initial metal concentration of 10 mg/L (Table 2 and Fig. 4b).

Following initial metal concentration, the initial solution pH showed a strong significant effect (Tables 2 and 5, Fig 4a) on Cu(II) biosorption, which can be easily explained based on the fact that the positively charged Cu(II) ion require negatively charged surface for easy chelation[26]. Therefore, at a low pH,Cu(II) removal was also low due to competition between protons (H^+) and the Cu(II) ions for the same binding site on AT-NM. This is, however, reversed at a high pH due to decrease in H^+ ions, thereby favouring Cu(II) sorption onto the negatively charged biosorbent surface[38]. With respect to the effect of biosorbent dosage on Cu(II) biosorption, a maximum removal of 86% was observed at 1 g/L. (Fig. 4c and Table 2)also revealed that an increase in biosorbent dosage enhance the Cu(II) removal efficiency which can be explained due to the presence of a large number of functional groups with an increase in the biosorbent dosage. However, when the biosorbent dose was increased beyond a certain limit, the Cu(II) removal slightly decreased probablydue to a reduction in the availability of biosorbent binding sites as a result of crowding effect or aggregation of AT-NM thus causing a reduction in the biosorbentsurface area[39].



(a) (b)

(c)

Fig.4. Response surface plot showing the interaction between different process parameters on Cu(II) biosorption by AT-NM: (a) initial solution pH and initial metal concentration, (b) AT-NM biosorbent dosage and initial metal concentration, (c) AT-NM biosorbent dosage and pH.

At the RSM optimized values of initial solution pH of 5.24, initial metal concentration of 17.33 mg/L, biosorbent dosage of 1.1 g/L a 3% increase in the Cu(II) removal was achieved, which was also verified by carrying out an experiment at these optimized conditions. Hence, it could be well said that the BBD-RSM aided in achieving enhancement in the Cu(II) removal by AT-NM. However, in order to further establish the potential of AT-NM as a low cost biosorbent for heavy metal removal from wastewater, more studies aimed at investigating the desorption of bounded Cu(II) from the biosorbent, effect of co-ions on Cu(II) removal and dynamic Cu(II) removal in a packed bed reactor need to be performed.

5. Conclusions

AT-NM characterization using FTIR spectroscopy revealed a major involvement of N-H group for Cu(II) binding onto the biosorbent. FESEM and BET surface area analyses further revealed a very high porosity and surface area of AT-NM for an efficient binding of Cu(II) onto the biosorbent. The Cu(II) sorption mechanism onto AT-NM was confirmed by an accurate fit of thepseudo second-order kinetic model to the experimental data. The high affinity of Cu(II) to AT-NM was further established from the 1/n value of the best fitting Freundlich isotherm model. Weber and Morris model fitting of the kinetic experimental dataillustrated that maximum Cu(II) removal was due to its intraparticle diffusion. Among the different sorption parameters, initial metal concentration was the most significanton Cu(II) removal by AT-NM followed by initial solution

pH and AT-NM dosage. Thus, thepresent study demonstrated an efficient Cu(II) removal from aqueous solution by AT-NM under the RSM optimized process conditions.

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