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Research paper

Applications of Box–Behnken experimental design coupled with artificial neural networks for biosorption of low concentrations of cadmium using *Spirulina (Arthrospira) spp.*

R.R. Siva Kiran [a], G.M. Madhu [a,*], S.V. Satyanarayana [b], P. Kalpana [c], G. Subba Rangaiah [c]

[a] *Department of Chemical Engineering, MS Ramaiah Institute of Technology, Bangalore 560054, India*

[b] *Department of Chemical Engineering, JNTUA College of Engineering, Anantapur 515002, India*

[c] *Department of Botany, College of Science & Technology, Andhra University, Visakhapatnam 530003, India*

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Abstract

The present study deals with the application of artificial intelligence techniques coupled with Box–Behnken (BB) design to model the process parameters for biosorption of cadmium using live *Spirulina (Arthrospira) spp.* as adsorbent in open race way pond with Zarrouk medium. The biomass concentration of *Spirulina spp.* decreased to half at 4 ppm Cd (II) after 8 days. Based on the LCt50 values, 3.69 ppm (8th day), *Spirulina (Arthospira) maxima* showed maximum tolerance. Considerable growth and bioaccumulation of *Spirulina spp.* is observed below 1 ppm and tolerant up to 3 ppm. The cadmium adsorption on *Spirulina spp.* showed good correlation ($R^2 = 0.99$) when applied to Freundlich equation and data fit into pseudo second order kinetics. A four factorial, three blocks and three level Box–Behnken design with initial concentration (1 ppb to 5 ppb), biosorbant dosage (0.1 gdw to 0.2 gdw), agitation speed (12 rpm to 16 rpm) and pH (6 to 8) as independent variables and percentage adsorption as dependent variable were selected for study. The data were further processed using artificial neural network model and DIRECT algorithm for better optimization. The final Cd (II) concentration of <0.5 ppb was achieved with 1 ppb initial concentration under optimal conditions. A continuous desorption process was also developed for removal of cadmium from *Spirulina (Arthrospira) sp.*

Keywords: *Spirulina (Arthrospira) sp*; Bioaccumulation; Box–Behnken design; Artificial Neural Networks; DIRECT algorithm

1. Introduction

Biological adsorption or biosorption is accumulation of the heavy metal ions on microbial cell surfaces and also transported through protein carriers into the cellular interiors. Cadmium is one of the most toxic heavy metals found in drinking water. It is reported in most of the recent studies on heavy metal removal at low concentrations, the biological adsorption is more economical and superior when compared with other adsorption process [1–4].

In our previous experiments, bioaccumulation of cadmium on *Spirulina sp.* was studied along with biosorption capabilities [5–7]. Extensive studies on *Spirulina sp.* were carried out by Dunn et al. [8], on the management of noxious odor emissions in tannery waste stabilization ponds using microalgal capping. Balaji et al. [9,10] studied the toxicity of lead, chromium, cadmium, zinc and nickel on three different *Spirulina (Arthrospira) sp.* (*A. indica, A. maxima,* and *A. platensis*). Many studies reported on cadmium removal by biosorption and chemical adsorption in ppm level. Few studies are reported at ppb level due to the complexity of the analytical technique involved in determining very low metal concentrations.

Statistically based experimental designs like response surface methodology are more efficient in experimental biology, as variables are tested simultaneously [7]. Due to the sensitivity of the experimental studies and time taken for each experimental run to determine low concentrations at ppb level, it is necessary to minimize the total number of experimental runs by using advanced techniques like Response Surface

* Corresponding author. Department of Chemical Engineering, M.S. Ramaiah Institute of Technology, MSR Nagar, Bangalore, Karnataka, India. Tel.: +91 80 23600822; fax: +91 80 2360 3124.

E-mail address: gmmadhu@gmail.com (G.M. Madhu).

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Methodology (RSM) and Artificial Neural Networks (ANN) [11,12].

The present study deals with the application of artificial intelligence techniques coupled with Box–Behnken (BB) design to model the process parameters for biosorption of cadmium below 1 ppm using live *Spirulina (Arthrospira) sp. (A. indica, A. maxima and A. platensis)* as adsorbent in lab scale open race way pond (0.5 m long, 0.4 m wide and 0.075 m deep) with Zarrouk medium [13,14]. A continuous process is developed for desorption of cadmium from *Spirulina (Arthrospira) plantensis*.

2. Materials and methods

2.1. Microorganism and media composition

Spirulina (Arthospira) maxima, *Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* were procured from Center for Advanced Studies, University of Madras, Chennai, Tamil Nadu, India. The alga seeds were first centrifuged and then stored in Zarrouk medium [14] for 7 days at 20~26 °C under light generated by a 40 W white fluorescent lamp [5–7].

2.2. Bioaccumulation and toxicity studies

Experiments were carried out in 3 L open race way ponds (0.5 m long, 0.3 m wide and 0.075 m deep) with initial *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* biomass of 0.1 $gdw.L^{-1}$ in Zarrouk's medium containing 1 $mg.L^{-1}$ of Cd (II) ions with working height 0.03 m. The cultures were kept under 60 W light (Philips, India) at an intensity of 2000 lux with 12 h light/dark photoperiod at 30 °C ± 2 °C is maintained for 8 days. The pH of the medium was 10. Acute toxicity test parameters like Lethal Concentration and time (LCt50) of Cd (II) ions and percentage decrease in the *Spirulina.* biomass were calculated. Cell concentration was estimated at 560 nm [5,6] wavelength using UV-visible spectrophotometer (Shimadzu, Japan) and the same was used for finding the growth curve of *Spirulina (Arthospira) spp*.

2.3. Biosorption experiments

The biosorption of Cd (II) ions were carried out in 25 L open raceways (0.7 m long, 0.30 m wide, 0.25 m deep) containing separate cultures of *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* with an initial biomass concentration of 0.1 $g.L^{-1}$ for 24 h. The cultures were mixed using paddle wheels turning at 12 rpm and illuminated with daylight-type 40 W fluorescent lights (Philips, India) at an intensity of 1900 lux and a 12 h light/dark photoperiod at 30 °C with pH 10. The metal concentration was analyzed using Atomic Absorption Spectrophotometer (GBC Avanta Ver 1.32, Australia). For biosorption experimental studies, Lagergren's first order kinetics equation [15] and the pseudo second order rate equation suggested by Ho [16,17], Langmuir [18,19] adsorption isotherm and Freundlich adsorption isotherm equation [20] were applied.

2.4. Box–Behnken (BB) design and Artificial Neural Networks

Box–Behnken (BB) design with 4 factors each at 3 levels, 3 blocks and with 3 replicates at the center points, leading to 27 sets of experiments are used for modeling the process parameters for biosorption of cadmium at various initial concentration (1, 3 and 5 ppb), biosorbent dosage (0.1, 0.15 and 0.2 gdw), agitation speed (12, 14 and 16 rpm) and pH (6, 7 and 8). The total number of experiments is 81 and the standard error for 3 replicates at center points is found to be ±0.05. Extremely low concentrations in (ppb) of cadmium were estimated by chelation with ammonium I-pyrrolidinedithiocarbamate (APDC) and diethylammonium diethyldithiocarbamate (DDDC), a double extraction into chloroform, and back-extraction into nitric acid [21,22]. From the BB experiments, a second-order polynomial equation was fitted for adsorption (Eq. 1).

$$\begin{aligned} Y = {} & \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \\ & + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 \\ & + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 \end{aligned} \quad (1)$$

Where Y is the predicted response (% adsorption), β_0 the offset term, β_1, β_2, β_3 and β_4 the linear effect, β_{11}, β_{22}, β_{33} and β_{44} the squared effect, β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34} the interaction effect. The proportion of variance explained by the second order polynomial model was given by the multiple coefficient of determination, R^2. Analysis of variance (ANOVA) was performed using Statistica software (Version 6.0, by Stat Soft Inc., Tulsa, USA). The second order polynomial equation was further optimized for better multiple coefficient of determination using Artificial Neural Networks (ANN). A network topology was developed by trial and error method with different activation functions, training algorithms, training parameters, number of hidden layers, number of neurons in each hidden layer, initial weights, and training duration using Matlab (MathWorks, USA) software [23].

2.5. Desorption experiments

A Whatman filter paper 2 (8 micro pore size) was used to remove 0.8 g dry weight of *Spirulina* biomass rich in Cd (II) for desorption studies. The *Spirulina* biomass was added to a 250 ml conical flask containing 0.01 M NaOH and kept in orbital shaker at 120 rpm for 20 min. NaOH enhance the taste of *Spirulina sp.* [22] giving NaOH an advantage over using other cadmium desorption chemicals. The biomass was transferred to a separating funnel and kept under illumination of 2000 lux. After 2 h, the live *Spirulina* from the top layer was removed and the remaining bottom product was centrifuged at 5000 rpm and dried. The top layer containing *Spirulina* biomass was filtered and dried.

3. Results and discussion

3.1. Effect of Cd (II) ion on Spirulina (Athospira) spp.

The effect of Cd (II) ions on living cells of *Spirulina (Athospira) spp.* were found at various concentrations (0, 1, 2,

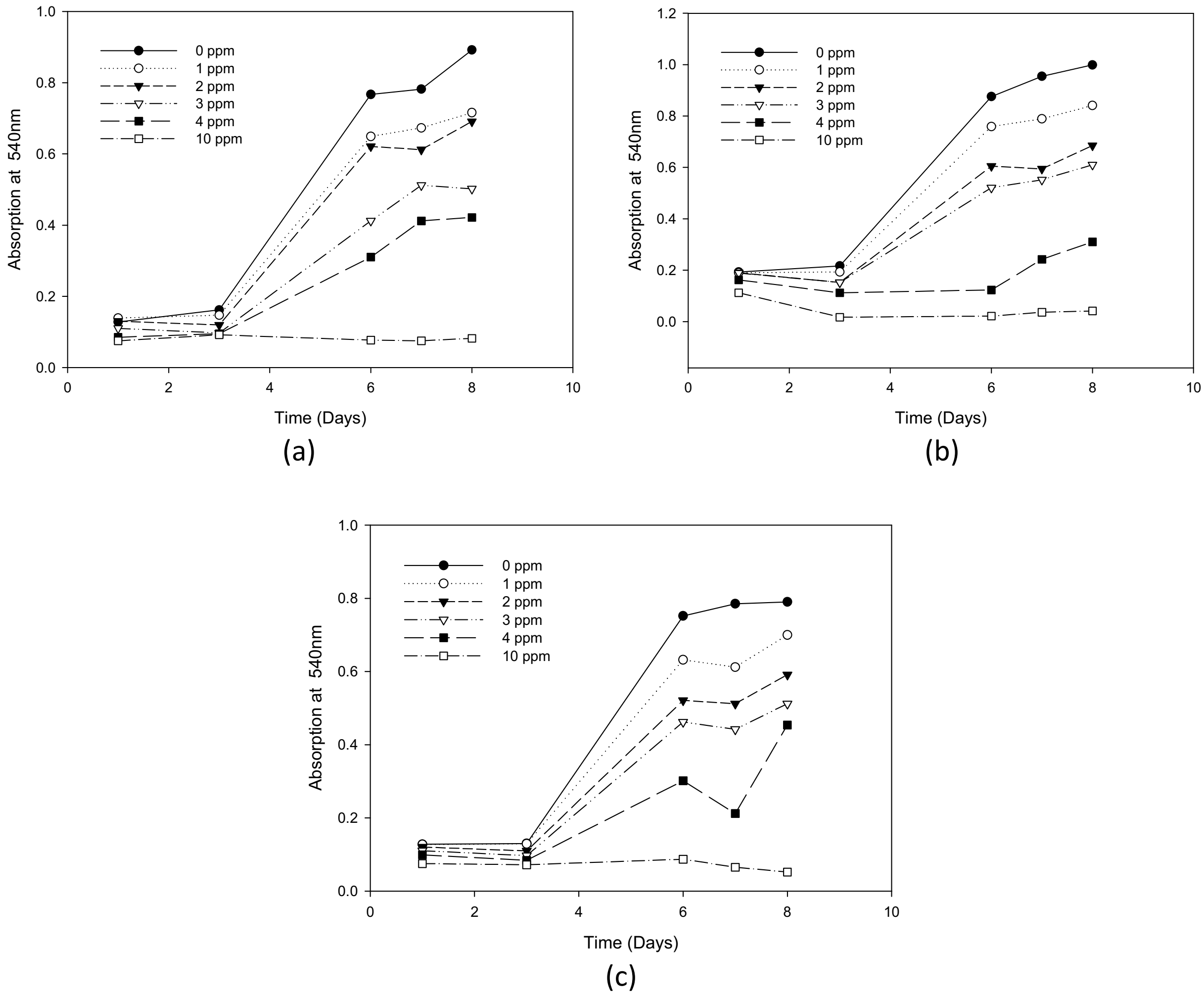

Fig. 1. Growth of *Spirulina (Arthrospira) maxima (a), Spirulina (Arthrospira) platensis (b) and c) Spirulina (Arthospira) indica* at various concentrations of Cd (II) ions in Zarrouk's medium (Zarrouk, 1966).

3, 4 and 10 ppm). The growth curves are shown in Fig. 1. It is evident that at 10 ppm Cd(II) concentration, the cadmium ions were found to be toxic. Considerable growth was observed below 4 ppm of Cd(II) for all three species. Cadmium is toxic to live *Spirulina* at concentrations greater than 4 ppm due to the bioaccumulation of cadmium into cellular interiors [6,7]. At low concentrations (i.e. less than 1 ppm) toxic effect of Cd(II) on all three *Spirulina spp*. were observed to be less and the species is tolerant at low concentrations. From the present work, it is evident that Cd (II) ions from low concentration contaminant wastes can be removed using *Spirulina (Athospira) sp*. The percentage decrease in the species and Lethal Concentration and time (LCt50) are tabulated in Table 1.

3.2. Study of contact time

Pseudo first order and pseudo second order kinetics were applied for predicting the adsorption rate and for finding the optimal equilibrium concentration and time. The effect of Cd (II) ion concentration on adsorption for different *Spirulina spp.* at pH 7 is shown in Fig. 2. The adsorption rate within the first 6 min was found to be very high and thereafter the adsorpion proceeded at a slower rate till equilibrium was reached.

The pseudo first order equation: The pseudo first order equation of Lagergren [15] is generally expressed as follows in Eq. 2.

$$dq_t/dt = k_1(q_e - q_t) \tag{2}$$

Pseudo second order equation is given by Eq. 3.

$$dq_t/dt = k_2(q_e - q_t)^2 \tag{3}$$

Where q_e and q_t are the sorption capacities (mg.gdw^{-1}) at equilibrium and at time *t* (min) and k_1 and k_2 are the rate constant for pseudo first order and pseudo second order kinetics. gdw is gram dry weight of *Spirulina (Athospira) sp* and the approximate dry weight used in the pilot scale open raceway pond was found to be 0.12 g. The equilibrium time was found to be 6 min with 67.9% removal of Cd (II) ions. Further, all process parameters were evaluated at 6 min.

The effect of Cd(II) concentration on adsorption using three *Spirulina sp.* was studied (Fig. 2). *Spirulina (Arthospira) indica* showed maximum adsorption capacity when compared with the other two species. The consistent metal uptake pattern was also observed in case of *Spirulina (Arthospira) indica*.

Table 1
Percentage decrease in the biomass of three Spirulina sp. grown in the presence of Cd (II) ions and LCt50.

Cd (II) Metal Conc. (ppm)	Algae	Percentage decrease in the productivity				LCt50
		1st day	3rd day	7th day	8th day	
1	*Spirulina indica*	99.21	99.23	77.96	88.58	03.21 ppm
2		93.82	84.30	65.22	74.79	(7th day)
3		86.09	74.38	56.30	64.79	
4		77.03	64.46	27.38	57.45	
1	*Spirulina platensis*	98.44	88.94	82.61	84.18	3.36 ppm
2		98.49	70.18	62.21	68.57	(7th day)
3		97.92	70.09	57.80	61.08	
4		93.93	51.61	25.34	31.05	
1	*Spirulina maxima*	99.27	90.74	86.06	80.26	3.69 ppm
2		94.27	73.82	78.26	77.46	(8th day)
3		79.85	59.87	65.47	56.27	
4		61.66	58.64	64.19	47.25	

From Fig. 2, it proves that the *Spirulina (Arthospira) indica* is potential adsorbent for adsorption of Cd (II) ions on the species. Till 10 min, consistent pattern was observed in case of *Spirulina (Arthospira) maxima* after 15 min, the adsorption rate is reduced. Below 15 min, the *Spirulina (Arthospira) maxima* can be used for biosorption of Cd (II) for period of less than 15 min. Complete equilibrium was reached after 30 min as shown in the Fig. 2. The kinetic constants for Cd (II) biosorption are tabulated in Table 2. The regression coefficient R^2 (0.999) clearly states that the data perfectly fit into the pseudo second order kinetics. Negligible deviation is observed between adjusted R^2 and R^2 values for pseudo first order kinetics equation. This confirms the fact that the predicted equation will represent the entire data range of individual points. In case of *Spirulina (Arthospira) platensis,* the data perfectly fit into first order predicted equation.

For *Spirulina (Arthospira) indica*, the deviation for pseudo first order equation is high and confirms that the equation does not represent the individual data points. The pseudo second order kinetic equation fits the data perfectly and the adjusted R^2 and R^2 values are similar, confirming the validity of the model. From the results, we can infer that less than 1 mg.L^{-1} of Cd (II) ions concentration, *Spirulina* can be used as adsorbent. (Fig. 3).

3.3. Biosorption equilibrium studies

The equilibrium time for biosorption of *Spirulina (Arthospira) maxima* and *Spirulina (Arthospira) platensis* was taken as 6 min and for *Spirulina (Arthospira) indica* was taken as 5 min from the kinetic studies (Fig. 2). The Langmuir and Freundlich models were fitted for the experimental data. The Langmuir isotherm (Eq. 4) can be expressed as

$$Q = Q_{\max} bC_f / (1 + bC_f) \quad (4)$$

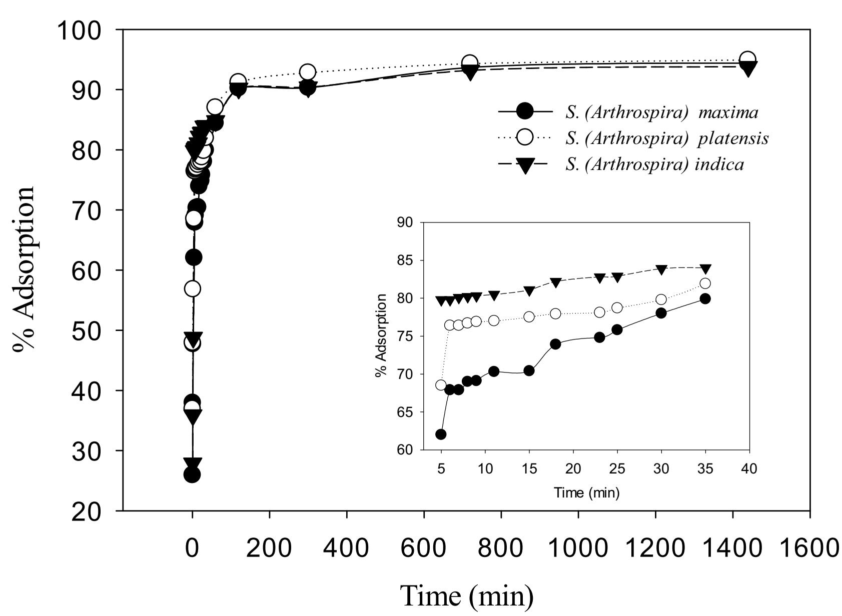

Fig. 2. The effect of time on Cd (II) biosorption using live *Spirulina (Arthospira) maxima*, *Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*.

Table 2
Kinetic constants for Cd (II) ions biosorption onto live *Spirulina (Arthospira) maxima*, *Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*.

Organism	Initial conc. (ppm)	Pseudo first order kinetics				
		k_I	q_e	R^2	Adjusted R^2	Equation
Spirulina (Arthospira) maxima	1	0.242	1.426	0.979	0.974	$\log(q_{eq}-q)=0.3552-0.1055t$
Spirulina (Arthospira) indica	1	0.360	1.382	0.951	0.939	$\log(q_{eq}-q)=0.3242-0.1565t$
Spirulina (Arthospira) platensis	1	0.394	1.46	0.999	0.999	$\log(q_{eq}-q)=0.3785-0.1714t$
		Pseudo Second Order Kinetics				
		k_{II}	q_e	R^2	Adjusted R^2	Equation
Spirulina (Arthospira) maxima	1	0.055	3.93	0.999	0.999	$t/q=1.1726+0.2540t$
Spirulina (Arthospira) indica	1	0.074	3.95	0.999	0.999	$t/q=0.8562+0.2527t$
Spirulina (Arthospira) platensis	1	0.087	3.90	0.999	0.999	$t/q=0.7476+0.2559t$

Where Q is monolayer adsorption capacity of adsorbent ($mg.gdw^{-1}$) and Q_{max} is maximum value of Q and *b* ($L.mg^{-1}$) is the Langmuir constant.

Freundlich Isotherm (Eq. 5) is given by

$$Q = KC_f^{1/n} \tag{5}$$

Where K($mg.g^{-1}$) is the Freundlich constant related to adsorption capacity of adsorbent and C_f ($mg.L^{-1}$) is equilibrium concentration of Cd (II) ions. The linearized Langmuir (Eq. 6) and Freundlich (Eq. 7) were given below

$$Q = 58.47C_f/(1+0.34C_f) \tag{6}$$

$$Q = 5.08C_f^{1/1.24} \tag{7}$$

The adsorption of cadmium (II) ions on the *S. (Arthrospira) maxima* and *S. (Arthrospira) indica* was found satisfying with the Freundlich equation as compared with the Langmuir equation. The results are tabulated in Table 3.

From the value of regression coefficient (R^2), normalized deviation (ND) and normalized standard deviations (NSD) [17], we can conclude that three species fit better to the Freundlich equation when compared with Langmuir isotherm. The separation factor (R_L), estimated using Langmuir model constants are within the range of 0 to 1, confirming the favorability of biosorption [17]. The deviation between adjusted R^2 and R^2 value are less in case of all three species suggesting that the model represents all individual data points in the experimental study. Solisio et al. [24] studied the biosorption of Cd (II) on *Spirulina platensis* and found that the adsorption capacity decreased from 357 $mg.g^{-1}$ to 149 $mg.g^{-1}$, when there is increase in percentage adsorption. The similar kind of pattern was observed in the present study (Table 4), where the adsorption capacity increased for all three species when there is a decrease in the adsorption percentage. *Spirulina* studies, including, Şeker et al. [25] on Cd (II) heavy metal ions fits better to Freundlich isotherm and Chojnacka et al. [26] on Cd (II) ions fits to Langmuir equation. Rangsayatorn et al. [27] studied Cd (II) adsorption on *Spirulina platensis* and fitted data into Langmuir equation. Doshi et al. [28], studied the adsorption of Cd (II) on live and dead *Spirulina* sp and found that the data fit into the Langmuir model.

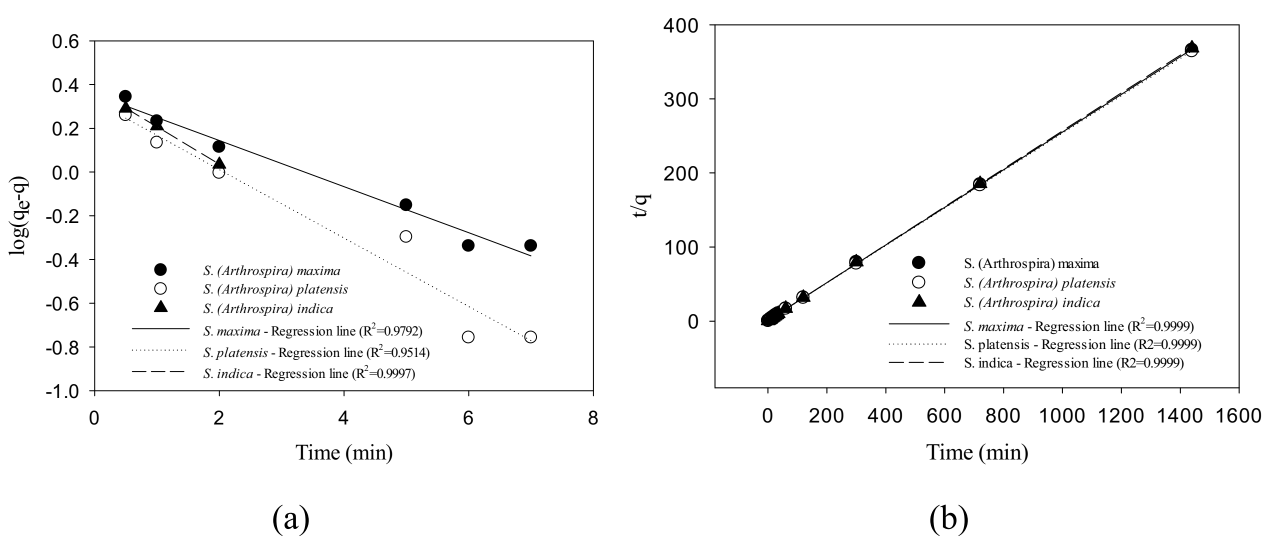

Fig. 3. Pseudo first order (a) and Pseudo second order (b) kinetics with regression line and multiple coefficient of determination (R) for Cd (II) biosorption.

Table 3
Langmuir and Freundlich constants and correlation coefficients for Cd (II) biosorption.

Organism	Adsorption isotherm		
S. (Arthrospira) maxima	Langmuir	b	0.325
	$q_{eq} = \frac{9.66C_{eq}}{1+0.3249C_{eq}}$	q_{max}	29.760
		R_L	0.754
		Correlation coefficient R^2	0.980
		Adjusted R^2	0.969
		ND	0.1424
		NSD	1.595
	Freundlich	n	1.240
	$q_{eq} = 6.3328C_{eq}^{0.8016}$	K_f	6.333
		Correlation coefficient R^2	0.999
		Adjusted R^2	0.998
		ND	11.38
		NSD	1.317
S. (Arthrospira) platensis	Langmuir	b	0.881
	$q_{eq} = \frac{16.10C_{eq}}{1+0.8808C_{eq}}$	q_{max}	18.280
		R_L	0.5316
		Correlation coefficient R^2	0.969
		Adjusted R^2	0.953
		ND	0.2413
		NSD	4.001
	Freundlich	n	1.567
	$q_{eq} = 8.3272C_{eq}^{0.6383}$	K_f	8.327
		Correlation coefficient R^2	0.996
		Adjusted R^2	0.994
		ND	0.04
		NSD	0.07
S. (Arthrospira) indica	Langmuir	b	1.110
	$q_{eq} = \frac{20.29C_{eq}}{1+1.11C_{eq}}$	q_{max}	18.280
		R_L	0.4739
		Correlation coefficient R^2	0.978
		Adjusted R^2	0.967
		ND	0.2939
		NSD	3.6139
	Freundlich	n	1.6025
	$q_{eq} = 9.5148C_{eq}^{0.6242}$	K_f	9.515
		Correlation coefficient R^2	0.986
		Adjusted R^2	0.979
		ND	0.080
		NSD	0.260

3.4. Experimental design and data analysis

The Box–Behnken experimental design is a very powerful tool to determine the optimal level of process parameters with less number of experiments when compared with other design of experiment models. In the preliminary step of optimization, the selected initial conc. of cadmium (II) ion at ppb level (parts per billion), biosorption dosage (gdw of *Spirulina* (*Athospira*) *maxima*), agitation speed (rpm) and pH were taken. Temperature was not considered as a parameter for the study because many open raceway ponds operate at normal room temperature and sun light as light source. The temperature of the present study is maintained constant at ambient temperature 25 ± 3 °C. The experimental design table showing different levels of process parameters, experimental values and predicted values, Second Order Polynomial Equation was fitted for the Box–Behnken Experimental Design is present in Table 5.

Table 4
Cadmium metal uptake q_{eq} (mg.g^{-1}) and percentage biosorption.

Biosorbent	Metal uptake q_{eq}(mg.g^{-1})	Adsorption %
Spirulina (Arthospira) maxima	2.833	68
	5.458	65.5
	7.792	62.33
	10.167	61
Spirulina (Arthospira) platensis	3.208	77
	6.000	72
	8.208	65.67
	10.625	63.75
Spirulina (Arthospira) indica	3.333	80
	6.417	77
	8.700	69.6
	11.163	66.98

Regression analysis of the experimental data was done and the following second order polynomial equation (Equation 1) shows the relationship between the percentage adsorption and the other process parameters that were present in Table 6.

Where Y is the predicted Cd (II) adsorption percentage and x_1, x_2, x_3 and x_4 are the coded terms for initial concentration (ppb),

Table 5
Experimental plan of the optimization design with the experimental and predicted values for the biosorption of Cd (II) ions on open raceway pond using *Spirulina (Arthospira) maxima*, *Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* as biosorbents.

x1	x2	x3	x4	% Adsorption								
				Spirulina (Arthospira) maxima			*Spirulina (Arthospira) platensis*			*Spirulina (Arthospira) indica*		
				Experimental	Predicted BB	Predicted ANN	Experimental	Predicted BB	Predicted ANN	Experimental	Predicted BB	Predicted ANN
1	0.10	14	7	68.00	66.79	68.00	73.23	74.90	73.23	78.68	79.51	78.68
5	0.10	14	7	58.45	60.68	58.45	62.32	62.21	61.65	65.25	64.39	65.13
1	0.20	14	7	73.25	70.95	73.25	79.23	79.41	79.23	82.45	81.15	82.45
5	0.20	14	7	60.32	61.46	57.85	65.42	63.82	65.42	63.71	66.03	63.71
3	0.15	12	6	58.32	57.50	58.32	62.75	63.28	62.75	66.54	67.41	69.31
3	0.15	16	6	56.63	56.81	56.63	61.4	61.31	61.4	68.4	68.32	68.4
3	0.15	12	8	57.35	57.10	57.35	63.14	63.30	63.6	67.41	65.95	67.41
3	0.15	16	8	57.15	57.90	57.15	62.41	61.94	62.41	65.21	66.86	65.21
3	0.15	14	7	61.32	61.60	62.72	65.89	65.61	65.64	69.62	69.66	69.71
1	0.15	14	6	65.35	66.09	63.53	76.41	75.80	71.23	79.42	79.58	79.42
5	0.15	14	6	61.23	60.43	61.23	61.01	60.35	61.01	65.14	64.46	65.14
1	0.15	14	8	67.74	68.59	67.74	74.22	74.82	74.22	78.42	78.12	78.42
5	0.15	14	8	59.32	58.63	59.32	61.45	61.99	61.45	61.78	63.00	61.78
3	0.10	12	7	61.31	60.62	61.31	64.18	63.84	64.18	66.72	67.33	66.72
3	0.20	12	7	63.32	63.55	63.32	66.85	67.43	66.85	69.99	68.98	69.99
3	0.10	16	7	61.32	61.13	57.6	63.34	62.70	63.34	68.32	68.25	68.32
3	0.20	16	7	62.41	63.14	62.41	64.97	65.24	64.73	69.01	69.89	69.07
3	0.15	14	7	63.41	63.22	62.72	65.61	65.86	65.64	69.79	69.66	69.71
1	0.15	12	7	65.32	67.03	65.32	76.78	75.39	76.78	78.14	78.68	78.14
5	0.15	12	7	59.14	58.95	59.14	62.75	63.20	62.75	63.1	63.56	63.1
1	0.15	16	7	66.60	66.81	66.6	76.12	75.67	76.12	79.52	79.59	79.52
5	0.15	16	7	60.95	59.27	60.95	58.21	59.59	58.21	66.92	64.47	66.92
3	0.10	14	6	61.32	61.59	61.32	61.54	61.67	61.54	68.12	68.23	68.12
3	0.20	14	6	63.12	63.56	63.12	64.75	65.45	64.75	70.25	69.88	70.25
3	0.10	14	8	61.85	61.44	62.56	63.41	62.71	63.41	67.39	66.78	67.39
3	0.20	14	8	64.65	64.41	64.65	65.19	65.06	65.19	68.93	68.42	68.93
3	0.15	14	7	63.42	63.33	62.72	65.41	65.43	65.64	69.58	69.66	69.71

Where, ×1: Initial Concentration (ppb); ×2: Biosorbent Dosage (gdw.L^{-1}); ×3: Agitator Speed (rpm); ×4:pH; BB-Box–Behnken Experimental Design; ANN: Artificial Neural Networks.

biosorbent dosage (gdw.L^{-1}), agitator speed (rpm) and pH respectively. The deviation between R^2 and adjusted R^2 was reported to be very less for *Spirulina (Arthospira) indica* when compared with the other two species. These values signify that the model equation predicted for *Spirulina (Arthospira) indica* is more accurate and represents the actual data points and the deviation is also found to be less when compared with other two species. The above equations were also analyzed by plotting response surface contour plots and ANOVA (Analysis of Variance) (Table 7).

The coefficient of determination (R^2) from the experimental trails is found to be very low value and it can be further improved by theoretical modeling. For better R^2 value and for better optimization, artificial neural networks was employed to process the above data.

The configuration of the network for *Spirulina (Arthospira) maxima* is found to be 4-9-1 (4 neurons in the input layer and 9 neurons in the hidden layer and 1 neuron in the output layer and $R^2 = 0.965$) and for *Spirulina (Arthospira) platensis* ($R^2 = 0.967$),

Table 6
Second order polynomial model for the biosorption of Cd (II) ions onto *Spirulina (Arthospira) maxima*, *Spirulina (Arthospira) indica* and in 25 L open raceway pond.

Organism	Second-order polynomial equation	R^2	Adj. R^2
Spirulina (Arthospira) maxima	$Y = -124.514 - 0.510x_1 - 107.450x_2 + 15.743x_3 + 24.638x_4 + 0.521x_1^2 + 515.667x_2^2 - 0.600x_3^2 - 1.872x_4^2 - 8.450x_1x_2 + 0.033x_1x_3 - 0.537x_1x_4 - 2.3x_2x_3 + 5x_2x_4 + 0.186x_3x_4$	0.889	0.760
Spirulina (Arthospira) platensis	$Y = -78.7928 - 7.7740x_1 + 116x_2 + 8.9142x_3 + 25.8112x_4 + 1.0701x_1^2 + 76.1667x_2^2 - 0.3127x_3^2 - 1.9033x_4^2 - 7.25x_1x_2 - 0.2425x_1x_3 + 0.3287x_1x_4 - 2.60x_2x_3 - 7.15x_2x_4 + 0.0775x_3x_4$	0.982	0.962
Spirulina (Arthospira) indica	$Y = -108.159 - 6.410x_1 + 158.8x_2 + 12.148x_3 + 27.392x_4$ $0.758x_1^2 + 28x_2^2 - 0.281x_3^2 - 1.406x_4^2 - 13.275x_1x_2 + 0.153x_1x_3 - 0.295x_1x_4 - 6.450x_2x_3 - 2.950x_2x_4 - 0.508x_3x_4$	0.988	0.974

Table 7
Analysis of variance (ANOVA) for the four factorial Box–Behnken Experimental design for *Spirulina (Arthospira) maxima*, *Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* for Cd (II) ions biosorption.

Organism	Source of variation	SS	DF	MS	F-Ratio	p-value
Spirulina (Arthospira) maxima	x_1 & x_1^2	206.049	2.000	103.025	30.045	0.000
	x_2 & x_2^2	27.166	2.000	13.583	3.961	0.048
	x_3 & x_3^2	30.685	2.000	15.342	4.474	0.035
	x_4 & x_4^2	19.056	2.000	9.528	2.779	0.102
	$x_1 * x_2$	2.856	1.000	2.856	0.833	0.379
	$x_1 * x_3$	0.070	1.000	0.070	0.020	0.889
	$x_1 * x_4$	4.623	1.000	4.623	1.348	0.268
	$x_2 * x_3$	0.212	1.000	0.212	0.062	0.808
	$x_2 * x_4$	0.250	1.000	0.250	0.073	0.792
	$x_3 * x_4$	0.555	1.000	0.555	0.162	0.695
	Error	41.148	12.000	3.429		
	Total	372.279	26.000			
Spirulina (Arthospira) platensis	x_1 & x_1^2	697.395	2.000	348.697	288.231	0.000
	x_2 & x_2^2	28.376	2.000	14.188	11.728	0.002
	x_3 & x_3^2	16.678	2.000	8.339	6.893	0.010
	x_4 & x_4^2	19.641	2.000	9.821	8.118	0.006
	$x_1 * x_2$	2.103	1.000	2.103	1.738	0.212
	$x_1 * x_3$	3.764	1.000	3.764	3.111	0.103
	$x_1 * x_4$	1.729	1.000	1.729	1.429	0.255
	$x_2 * x_3$	0.270	1.000	0.270	0.224	0.645
	$x_2 * x_4$	0.511	1.000	0.511	0.423	0.528
	$x_3 * x_4$	0.096	1.000	0.096	0.079	0.783
	Error	14.517	12.000	1.210		
	Total	845.556	26.000			
Spirulina (Arthospira) indica	x_1 & x_1^2	735.081	2.000	367.540	446.165	0.000
	x_2 & x_2^2	8.128	2.000	4.064	4.933	0.027
	x_3 & x_3^2	9.223	2.000	4.611	5.598	0.019
	x_4 & x_4^2	16.898	2.000	8.449	10.256	0.003
	$x_1 * x_2$	7.049	1.000	7.049	8.557	0.013
	$x_1 * x_3$	1.488	1.000	1.488	1.807	0.204
	$x_1 * x_4$	1.392	1.000	1.392	1.690	0.218
	$x_2 * x_3$	1.664	1.000	1.664	2.020	0.181
	$x_2 * x_4$	0.087	1.000	0.087	0.106	0.751
	$x_3 * x_4$	4.121	1.000	4.121	5.002	0.045
	Error	9.885	12.000	0.824		
	Total	827.765	26.000			

DF: Degree of freedom; SS: Sum of squares; MS: mean squares ($SS.DF^{-1}$); MSE: Mean Square Error; F: F-Statistics ($MS.MSE^{-1}$), p-value: The probability of the actual event observed, together with any other equally extreme or more extreme events that might have occurred and for the above experimental data, p-value was calculated by Statistica v7.0 (Statsoft, USA).

it is found to be (4-17-1) and for *Spirulina (Arthospira) indica* ($R^2 = 0.9955$), it is (4-13-1). It is clearly observed that the number of neurons in the hidden layers is changing based on the experimental data and the complexity involved. The complexity of the data for *Spirulina (Arthospira) maxima* is less when compared with other two species. Experiments were performed to validate the data resulting from the predicted model as the neural networks predictions are completely empirical in nature.

Ravi et al. [29] have suggested an equation (Eq. 8) which was used for calculation of weights and bias using neural network. At maximum R^2 value, the weights and bias are used for optimization using DIRECT Algorithm suggested by Jones et al. [30].

$$y = w_2 * \left(2 \Big/ \left(1.0 + e^{-2/\left(w_1 * xv^1 + b_1\right)} - 1\right)\right) + b_2 \tag{8}$$

Where w_1 and w_2 are the weights, b_1 and b_2 are the biases. '**y**' is the predicted value from the neural network and **xv** is the row vector of 4 independent variables (x_1, x_2, x_3 and x_4), while $\mathbf{xv^1}$ represent the transpose of the vector with a dimension of (4×1). The above equation (Eq. 8) was uploaded into DIRECT algorithm for optimization.

A graph is drawn between the experimental (% Adsorption) and predicted (% Adsorption) values for all three species for Cd (II) ion biosorption for comparing the results from ANN and second order polynomial equation (Eq. 1) shown in Fig. 4. The graph clearly shows the superiority of the ANN when compared with second order polynomial equation for Cd (II) biosorption. The black circle represents the predicted Cd (II) % biosorption using second order polynomial equation and white circle represents the ANN. The values predicted by ANN are close to the regression line when compared to the values predicted by second order polynomial equation.

Fagundes-Klen et al. [31] applied ANN to the adsorption data and compared with Langmuir and Freundlich isotherms for Zn and Cd (II) adsorption using *Sargassum filipendula*

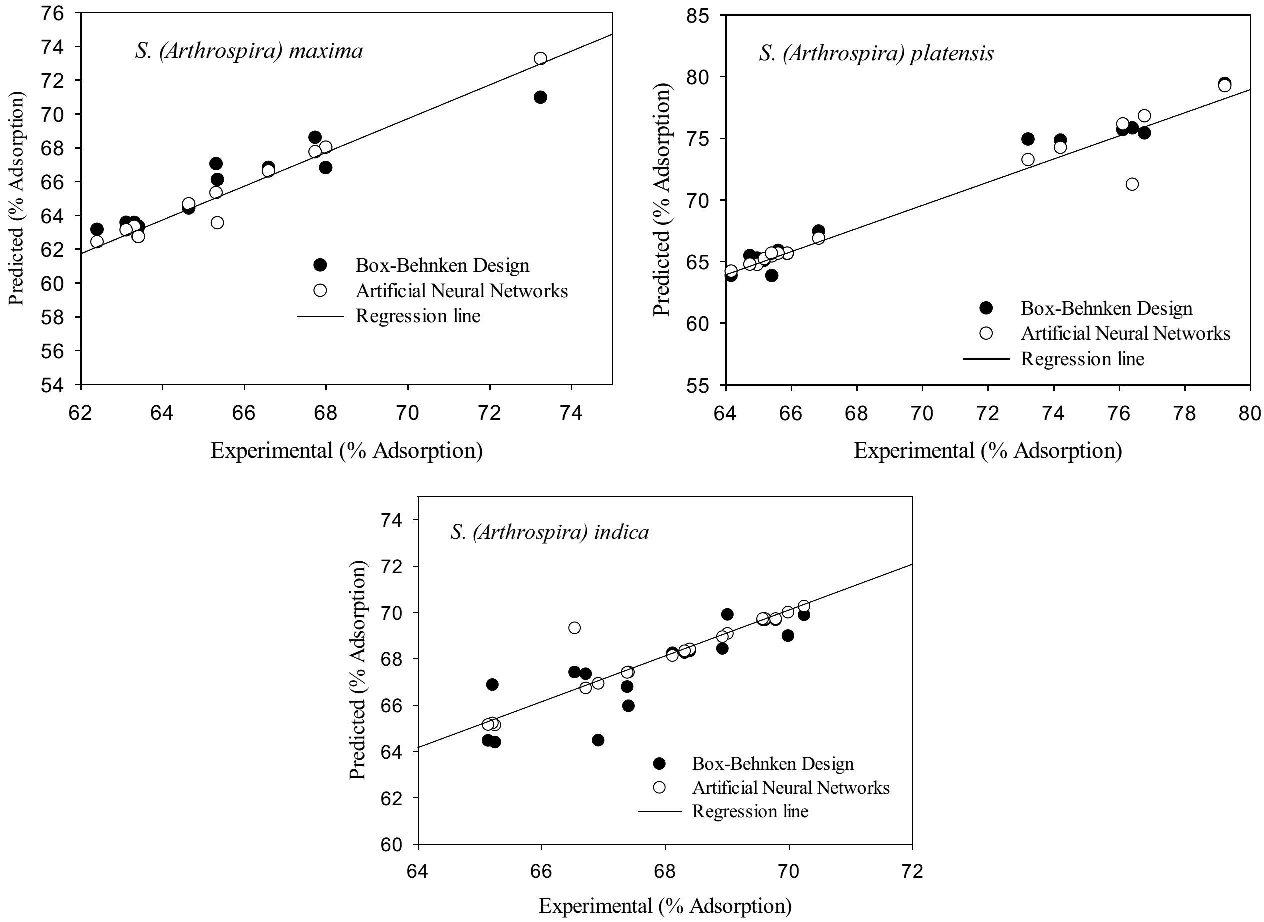

Fig. 4. Comparision of the predicted Cd (II) percentage adsorption and experimental Cd (II) percentage adsorption for Box–Behnken design and artificial neural networks for *Spirulina (Arthospira) spp.*

species. Fig. 4 was drawn between experimental and calculated equilibrium adsorption capacities and found that the neural network predictions are more accurate and near to the regression line when compared with Langmuir and Freundlich isotherms suggesting the superiority of the neural networks in predicting complex biosorption systems. The optimal values were predicted by DIRECT optimization are tabulated in Table 8.

The DIRECT algorithm calculates all possible optimized values based on our requirements and selects the maximum level of the process parameter for maximum percentage adsorption. The DIRECT Algorithm coupled with artificial neural intelligence had successfully predicted the values and the predicted response is high when compared with any value in the experimental result (Table 8) for all three species for Cd (II) biosorption. An experiment was performed at the optimal conditions predicted by the ANN and the Cd (II) ions on three *Spirulina sp.* (Table 8). The difference between the predicted and experimental was found to be less confirming the validity of the prediction as well as the artificial neural network model for Cd (II) biosorption. Sophisticated methodologies like design of experiments, response surface methodology, Box–Behnken experimental designs when coupled with ANN help in better predictions when compared with preliminary runs or adsorption isotherms. The optimal concentration of 0.25 ppb was achieved from 1 ppb initial concentration, 0.2 gdw *Spirulina maxima*, and 0.37 pbb for *Spirulina (Arthospira) platensis* and 0.14 ppb for *Spirulina (Arthospira)*

Table 8
Optimized values predicted by global optimization algorithm coupled with ANN.

Organism Name	Optimized values based on the model equation					
	Initial concentration (ppb)	Biosorbent dosage (gdw.L^{-1})	Agitator speed (rpm)	pH	% Adsorption	
					Predicted	Experimental
Spirulina (Arthospira) maxima	1.025	0.199	12.025	6.333	73.410	74.830
Spirulina (Arthospira) platensis	2.334	0.198	15.851	7.727	83.417	83.850
Spirulina (Arthospira) indica	1.003	0.103	14.658	7.922	84.720	85.730

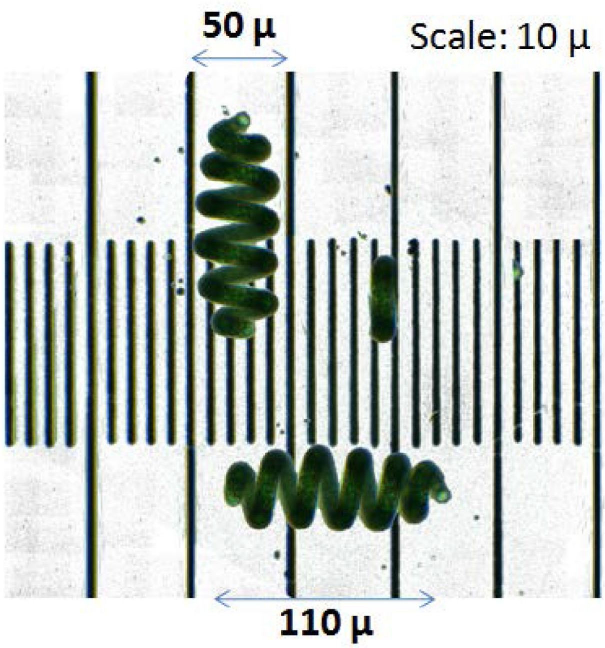

Fig. 5. *Spirulina sp.* size at 40× resolution.

indica at corresponding optimized conditions is given in Table 8.

3.5. Desorption experiments

Spirulina (Arthospira) platensis was considered for desorption experiment. *Spirulina* size was found using microscope (glass slide with scale) and photo editing software (Fig. 5). Based on the size of *Spirulina sp.* (10 µ), Whatman filter paper grade 2 was selected for separation.

The approximate cadmium content in 0.8 gdw *Spirulina* biomass was estimated to be 0.009 mg Cd (II). The equilibrium time for 85% desorption of Cd (II) using NaOH is 20 min [32,33]. The final Cd (II) concentration in the solution after 24 h is found to be 0.00034 mg Cd (II) in 150 ml (2.26 ppb) with percentage desorption of 96.22% (Fig. 6).

4. Conclusion

Bioaccumulation and biosorption potential of cadmium on live *Spirulina spp.* were successfully studied in open raceway ponds and LCt50 values were found. Box–Behnken experimental design coupled with artificial neural networks was successfully applied to biosorption of low concentrations of cadmium ion using live *Spirulina sp.* The *Spirulina* powder obtained from this process during desorption contains very less cadmium which can be further used as animal feed or plant bio-fertilizer. The process can also be tested using other species of *Spirulina sp.* for further increase of yield.

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Conflict of interest

The authors have no conflicts of interest to declare.

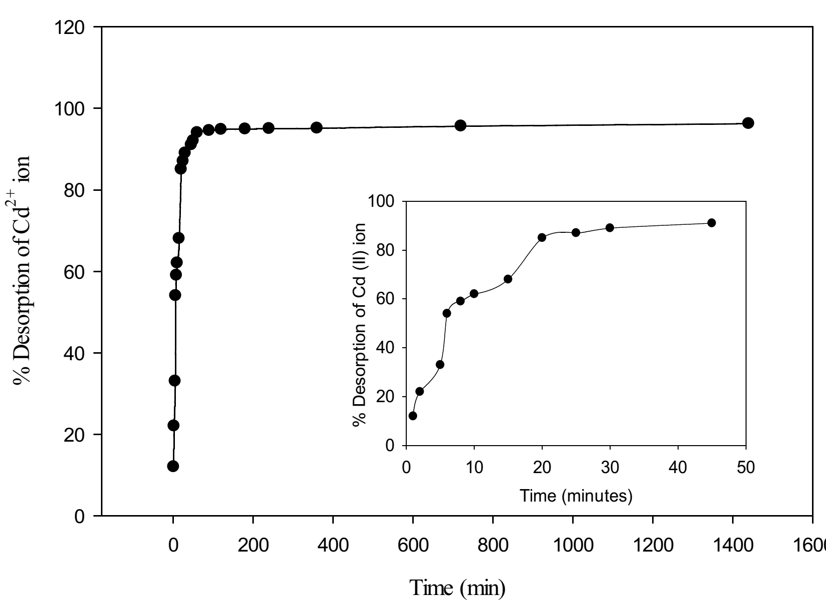

Fig. 6. Desorption kinetics of Cd (II) from *Spirulina (Arthrospira) platensis (a) platensis* biomass and total number of possible desorption cycles for biosorption of Cd (II) using *Spirulina (Arthrospira) platensis* biomass.

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