J Biochem Tech (2015) 6(1): 894-909
ISSN: 0974-2328

Equilibrium and kinetic studies of lead biosorption by three *Spirulina (Arthrospira)* species in open raceway ponds

Siva Kiran RR, Madhu GM*, Satyanarayana SV, Kalpana P, Bindiya P, Subba Rangaiah G

Received: 11 July 2015/ Received in revised form: 25 July 2015, Accepted: 08 August 2015, Published online: 10 August 2015

Abstract

The present investigation deals with the biosorption of lead from aqueous solutions in open race way pond using edible and live *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica, Spirulina (Arthospira) platensis*. Studies on various initial lead (II) ion concentrations, biosorbent dosage, pH and bioaccumulation potential were evaluated. The organisms are tolerant up to 4 mg/l and after that slight growth inhibition was found. *Spirulina (Arthospira) indica* showed more tolerance when compared with *Spirulina (Arthospira) maxima* and *Spirulina (Arthospira) plantensis*. The adsorption rate data was fitted to pseudo second order kinetics. The Langmuir and Freundlich models were applied to the experimental data and their equilibrium parameters were determined. Further optimization of initial lead (II) ion concentration, solution pH, agitation speed and biosorbent dosage were done using Box-Behnken experimental design coupled with artificial neural networks. This study provides a deep insight for exploring potential of using algal open race way ponds for biosorption of heavy metals. The diversity of the results can be expanded still further for other algal species and heavy metals.

Keywords: *Spirulina sp.*, Artificial Neural Networks (ANN), DIRECT algorithm, Box-Behnken design

Siva Kiran RR, Madhu GM*

Department of Chemical Engineering, MS Ramaiah Institute of Technology, Bangalore-560054, India

*Email: gmmadhu@gmail.com

Satyanarayana SV

Department of Chemical Engineering, JNTUA College of Engineering, Ananthapuramu-515002, India

Kalpana P, Bindiya P, Subba Rangaiah G

Department of Botany, College of Science & Technology, Andhra University, Visakhapatnam-530003, India

Introduction

Excessive use of heavy metals has seriously affected the environment and biodiversity of human, plant and animal population. Lead pollution has been considered as a major risk to air, soil and water. Batteries, pigment, plating, ammunition, rolled extrusions and cable sheathing are some industries responsible for release of lead in waste waters. Lead is a neurotoxic metal and its poisoning can cause severe mental or physical impairment. The recommended lead concentration by World Health Organization (WHO) in industrial waste streams is 10 μg/l (WHO 2011).

The recovery of lead from waste waters will become increasingly important to avoid further damage to the environment. Advanced techniques should be used with available resources for removal of lead from wastewaters before releasing to the water bodies. The use of microorganisms in the heavy metal removal has well documented since 19th century (Harris 1954; Dole 1952). Microorganisms, especially with their excellent surface properties and microscopic size are suitable targets for adsorption of heavy metal ions from solutions. The biological adsorption process has more advantages, when compared with conventional methods like chemical oxidation-reduction, precipitation, complexation, electrochemical operation, solidification, evaporation, adsorption, stabilization, vitrification, and ion exchange. The biosorption process can be highly selective, always provide with more surface area and easy adsorbent preparation, efficient and cost effective (Peters and Shem 1993; Puranik and Paknikar 1997; Puranik and Paknikar 1999).

The selective microorganism should always be safe for health and should be readily available. *Spirulina sp.* are multi-cellular and filamentous blue-green algae that had gained considerable popularity in the health and food industry. It is used as a protein and vitamin supplement to human, animal and aquaculture diets. It grows in Zarrouk's medium (Zarrouk et al. 1966) and can be harvested and processed easily and has a very high macro and micro-nutrient contents. Commercially, *Spirulina* alga is grown in outdoor raceway ponds in Earthrise farms, USA and Parry *Spirulina*, India. A raceway pond is an artificial pond with depth not more 4 feet and with varied length and breadth and can be used for the cultivation of algae in commercial scale. Presently, these open raceway ponds are also gaining popularity in algal biodiesel production (Rogers et al. 2014).

Biosorption using algae is considered to be easy when compared with other biosorption materials due to its availability and cost effectiveness. Chen and Pan (2004) studied the possibility of using live *Spirulina* to biologically remove aqueous lead below 50 mg/l from wastewater. Many studies on evaluating biosorption potential on pretreated or dead *Spirulina* biomass (Gong et al. 2005; Şeker et al. 2008; Solisio et al. 2006; Dotto et al. 2012; Babu et al. 2015; Markov et al. 2015; Kwak et al. 2015; Mitrogiannis et al. 2015; Deniz and Kepekci 2015) were available but very few biosorption studies were done on live *Spirulina sp* (Chen and Pan 2005; Hegde et al. 2015).

Statistical optimization techniques are powerful tools for handling complex data. Great quantities of experimental runs can often be reduced to more manageable proportions by performing of a few experiments which characterize the whole pattern of events and represents the entire set of combinations of factors. This can be achieved by applications of design of experiments. The experimental designs deal with the methods of analyzing variations in several factors simultaneously resulting in less number of experimental runs (Bailey Norman 1995). Box - Behnken experimental design can be easily applied to optimize variables when compared to factorial or fractional factorial designs. They require few experiments and effectively move through the experimental domain (Box and Behnken 1960). The results from the Box - Behnken experimental design were modeled using artificial neural networks and optimized using DIRECT (DIviding RECTangles) algorithm (Jones et al. 1993). Artificial neural networks (ANNs) are gaining importance due to the increase in the computational capacities and user friendly software technologies (Matlab, Mathworks, USA, Statistica, Statsoft, USA). The present work deals with the application of highly parallel computation based on distributed representation using Artificial Neural Networks (ANN) to solve and improvise the fitness of the data from the Box-Behnken experimental design and there by optimizing using DIRECT algorithm. These kind of advanced algorithms were rarely applied on complex biosorption data involving the adsorption of lead (II) ions using edible and live *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica,* and *Spirulina (Arthospira) platensis* in open raceway ponds.

Materials and Methods

Microorganism and media composition

Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica, Spirulina (Arthospira) platensis were procured from Center for Advanced Studies, University of Madras, Chennai, Tamil Nadu, India and OfERR, Chennai. The organisms was first filtered and concentrated to 0.1 g/l and then inoculated in Zarrouk's medium (Table 1) and maintained for 7 days at 20~26˚C under light generated by a 40 W white fluorescent lamp (Siva Kiran et al. 2012). The media components were added every 7 days to the culture flasks and water was replaced every 30 days for alga seed maintenance. Every two days, the alga seeds were monitored in the microscope for any contamination. pH of the solution was adjusted using sodium hydroxide and sulfuric acid. All chemicals used in study were procured from Merck, Germany (AR Grade) and the conductivity of the distillated water for chemicals preparation is 0.5 µmhos/cm.

Culture conditions

The *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica, Spirulina (Arthospira) platensis* growth characteristics were monitored by finding the optical density at 560 nm wavelength using UV-visible spectrophotometer (Shimadzu, Japan) at regular intervals. The biomass weight in grams was found by filtering *Spirulina (Arthospira)* species through Whatman No. 2 (8 µm) filter paper and dried at 80^0C for 4 h to 5 h until the sample reaches constant weight. The dry weight of the cell was determined after drying. Experiments were carried out in an open raceways pond (0.5 m long, 0.3 m wide, 0.075 m height) containing 3 l of *Spirulina (Arthospira)* cultures with an initial biomass concentration of 0.1 gdw/l. The cultures were illuminated with daylight-type 60 W fluorescent lights (Philips, India) at an intensity of 2000 lux in a 12 h light/dark photoperiod at 30˚C±2˚C. Lethal Concentration and time (LCt50) of lead (II) ion (Siva Kiran et al. 2012) were also calculated. Open raceway pond containing 3 L Zarrouk's medium with different concentrations of lead (II) ions (0, 1, 2, 4, 10 and 20 mg/l) and three *S. (Arthrospira)* culture (0.1 gdw/l) at 1:10 ratio were used for the growth and acute toxicity study and initial solution pH was measured to be 9. The growth rate was determined every 24 h for eight days in pond at each concentration and for each species (Siva Kiran et al. 2012).

Table 1: Composition of Zarrouk's Medium (Chen and Pan 2005)

Constituents	Concentration (g/L)
EDTA	0.08
$CaCl_2.2H_20$	0.04
NaCl	1.00
$NaNO_3$	2.50
$NaHCO_3$	16.80
$FeSO_4.7H_2O$	0.01
$MgSO_4.7H_2O$	0.20
K_2SO_4	1.00
K_2HPO_4	0.50

Biosorption experiments

Lead solutions of the required concentrations were prepared by dissolving the exact quantities of $PbNO_3$, respectively, in distilled water. About 25 l of lead (II) solution was prepared by diluting standard $PbNO_3$ solution to the desired concentrations. The biosorption experiments were conducted in another open raceway pond (0.7 m long * 0.3 m wide and 0.25 m height) with 25 l of freshly prepared lead solutions, with initial concentration 1 mg/l for 24 hours and biosorbant 0.24 g/l of *Spirulina (Arthospira) maxima*. Separate set of experiments with the same conditions were done by changing the organism to *Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*. Experiments were carried out to find the equilibrium time for optimal metal removal at 1 mg/l initial lead (II) concentration. Pseudo first order and pseudo second order kinetics were applied for modeling the biosorption process. Experiments were carried out to calculated equilibrium time. The open raceway pond was maintained at ambient temperature (30 ± 2˚C), agitator speed at 12 rpm, and pH was maintained at 7. To maintain the live culture in the biosorption medium, light intensity of 2000 lux was provided. To determine the concentration of the metal ions, the culture in the sample solutions was removed by filtration using Whatman No. 2 (8 µm) filter paper. The resulting solution was analyzed to measure the lead (II) concentration using Atomic Absorption Spectrophotometer (GBC Avanta Ver 1.32, Australia).

Pseudo first and Pseudo second order kinetics equation

Adsorption kinetics depends on process conditions, and heavy metal ions-adsorbent interactions. The kinetics should explain the mechanism and the reaction rate of adsorption system. The first adsorption kinetics of liquid-solid systems was explained by Lagergren (1898).

Legergren's first order kinetics equation

$$\log(q_{eq} - q) = \log q_{eq} - \frac{K_I}{2.303}t \qquad (1)$$

Where, q (mg/gdw) and q_{eq} (mg/gdw) is the adsorption capacity at time t (min) and at equilibrium respectively. K_I (mg-min/gdw) is the rate constant of pseudo first order kinetics. The pseudo first order system was applied to many biosorption experiments (Kalyani et al. 2009; Cheng et al. 2015; Anirudhan and Ramachandran 2015).

The pseudo second order rate equation was applied by Ho (1995) for explaining the kinetics of adsorption of divalent metal ions onto peat. The pseudo second order rate expression which was derived based on the above mechanism (Ho 1995; Ho and Mckay 1998; Ho 2004) was given by

$$\frac{t}{q} = \frac{1}{K_{II}{q_{eq}}^2} + \frac{1}{q_{eq}}t \qquad (2)$$

Where, K_{II} (gdw/mg-min) is the pseudo second order rate constant, q and q_{eq} are the adsorption capacity at time t (min) at equilibrium. A graph can be drawn between t/q and t to obtain the constants K_{II} and q_{eq}.

The present adsorption system contains adsorbent as live *Spirulina sp*, which is a blue green algae. *Spirulina* cell wall is composed of diverse array of microfibrillar polysaccharides embedded in matrix polysaccharides and proteoglycans, crystalline polymers interacting with various ions and water (Domozych 2011). The nature of the bonding between algal cell wall and metallic ions was explained by Crist et al. (1981). Due to the complex nature of the cell walls of the algal species, both covalent and ionic charge bonding may exist. The amino acids present in the proteins may provide amino, carboxy and sulfate functional groups which may be responsible for the adsorption of metal ions. These bonds are similar to the bonds that explained by Ho (1995) for peat biosorption and thus it justifies the application of the pseudo second order rate expression to the present system.

Biosorption equilibrium studies

Langmuir (1918) adsorption isotherm for physical adsorption is given by the expression

$$q_{eq} = \frac{q_{\max} b C_{eq}}{1 + bC_{eq}} \qquad (3)$$

Where, q_{max} (mg/gdw), indicates the monolayer adsorption capacity, 'b' (l/mg) the Langmuir constant and q_{eq} is the equilibrium adsorption capacity at metal ion equilibrium concentration C_{eq} (mg/l). The above equation can be rearranged to the following linear form:

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{bq_{\max}} + \frac{1}{q_{\max}}C_{eq} \qquad (4)$$

A graph between C_{eq}/q_{eq} and C_{eq} can be drawn, where b and q_{max} can be determined using slope and intercept.

An empirical relation between the concentration of the solute on the surface of an adsorbent and the concentration of the solute in the liquid was also given by Freundlich equation (1906) or Freundlich adsorption isotherm.

$$q_{eq} = K_f {C_{eq}}^m \qquad (5)$$

Where, K_f is Freundlich constant related to adsorption capacity of adsorbent and "m" relates to the adsorption intensity. The above equation can be simplified by applying logarithm on both sides.

$$\text{Log } q_{eq} = \log K_f + m \log C_{eq} \qquad (6)$$

A graph can be drawn between Log q_{eq} and and Log C_{eq} and the constants K_f (mg/gdw) and 'm' can be determined using slope and intercept (Limousin et al. 2007).

Box-Behnken experimental design and artificial neural networks

Response surface methodology is an empirical modeling technique for the evaluation of the relationship of a set of controlled experimental factors and observed results. A detailed account of this technique has been outlined as performing the statistically designed experiments, estimating the coefficients in a second order polynomial mathematical model using multiple regression, predicting the responses by solving the polynomial equation and finally validating the model by performing experiments at optimal levels of the factors. A 4-factor, 3 level Box-Behnken (BB) experimental design with 3 blocks and 3 replicates at the center points, leading to 27 sets of experiments, was used for the experimental study (Siva Kiran et al. 2010; Annadurai and Sheeja 1998). The initial concentration, biosorbant dosage, agitation speed and pH were chosen as the process variables and designated as x_1, x_2, x_3 and x_4 respectively. The low, middle and high levels of each factor were designated as -1, 0 and +1 respectively (Prakash et al. 2008). The Table 2 shows the levels of process variables for all three *Spirulina* species. (*Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica, Spirulina (Arthospira) platensis*). The equilibrium time at which the experiments were conducted for *Spirulina (Arthospira) maxima* was 7 minutes and for *Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* was 6 minutes from the initial studies. The experiments were conducted in open raceway pond (0.7 m long * 0.3 m wide and 0.25 m height) with working volume 25 l.

Table 2: Independent variables and their levels studied

Process Variables	Range studied	Levels of variables		
		-1	0	+1
x_1 (Initial Concentration, mg/l)	1-10	1	5	10
x_2 (Biosorbant Dosage, gdw/l)	0.1-0.2	0.1	0.15	0.2
x_3 (agitation speed, rpm)	12-16	12	14	16
x_4 (pH)	6-8	6	7	8

In a system involving four independent factors x_1, x_2, x_3 and x_4, a mathematical equation showing the relationship of the output response factor to each and every variable can be approximated by the quadratic (second degree) polynomial equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4$$

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. A multiple regression analysis is done to obtain the coefficients and the optimal values by solving four equations arising by differentiating the response variable with respect to x_1, x_2, x_3 and x_4 and equating them to zero. The significance of the fit explained by the polynomial models were given by the multiple coefficient of determination R^2. Analysis of variance (ANOVA) table was constructed to test the significance of the independent variables and their interactions (Yetilmezsoy et al. 2009). Statistica v6 (Stat Soft, USA) was used for this study.

The second order polynomial equation was further optimized for better multiple coefficient of determination and for determining the optimal values using Artificial Neural Networks (ANN) and global optimization technique. DIRECT algorithm developed by Jones et al. (1993) was implemented in Matlab Code by Tomlabs, USA (http://tomopt.com/tomlab/products/base/solvers/glbSolve.php) was used for this study (Hu et al. 2015).

ANN is applied to complex models such as adsorption data systems (Turan et al. 2011; Yetilmezsoy and Demirel 2008; Nagata and chu 2003; Ravi et al 2009). The Levenberg–Marquardt algorithm (LMA) (More Jorge 1978) based on back propagation model, as suggested by Kaan and Sevgi (2008), was selected after comparing various algorithms present in Matlab Software for biosorption data. The equation from the artificial neural networks was loaded into the DIRECT algorithm (Chiter 2006) for calculating the optimal values. Further experiment was done to find the validity of the model at optimal conditions predicted by DIRECT global optimization technique.

Results and discussion

Toxicity of lead (II) ion on three Spirulina (Arthospira) species

Live cells of *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica, Spirulina (Arthospira) platensis* were cultivated in Zarrouk's medium with various lead (II) concentrations (0, 1, 2, 4, 10 and 20 mg/l) for finding the toxicity limits. The pH of the medium solution was found to be 9. The growth curves were shown in Figure 1.

Figure 1 shows that lead (II) concentration at 20 mg/l is highly toxic for the growth of all three *Spirulina* species. Slight decrease in *Spirulina* live cells occurred at low lead (II) concentrations below 2 mg/l. *S. (Arthrospira) indica* is more tolerant when compared with other two species *Spirulina (Arthospira) maxima* and *Spirulina (Arthospira) platensis*. The growth curves plotted for *S. (Arthrospira) indica, Spirulina (Arthospira) maxima* and *Spirulina (Arthospira) platensis* at various concentrations of zinc and nickel, by Balaji et al (2014), *S. (Arthrospira) indica* showed more tolerance, when compared with *Spirulina (Arthospira) maxima* and *Spirulina (Arthospira) platensis*.

The percentage decrease in yield of *Spirulina* for all three species and the Lethal Concentration and time (LCt50) were tabulated in Table 3. The LCt50 value in mg/l was designated as 50% decrease in the productivity for 7 days. In case of *Spirulina (Arthospira) platensis* and *Spirulina (Arthospira) indica*, 50% decrease in the yield is beyond 20 mg/l lead (II) concentration at seventh day and for finding the LCt50 value, extrapolation of the data was done. The LCt50 value for *Spirulina (Arthospira) maxima* is in between 4 mg/l and 10 mg/l on seventh day. The

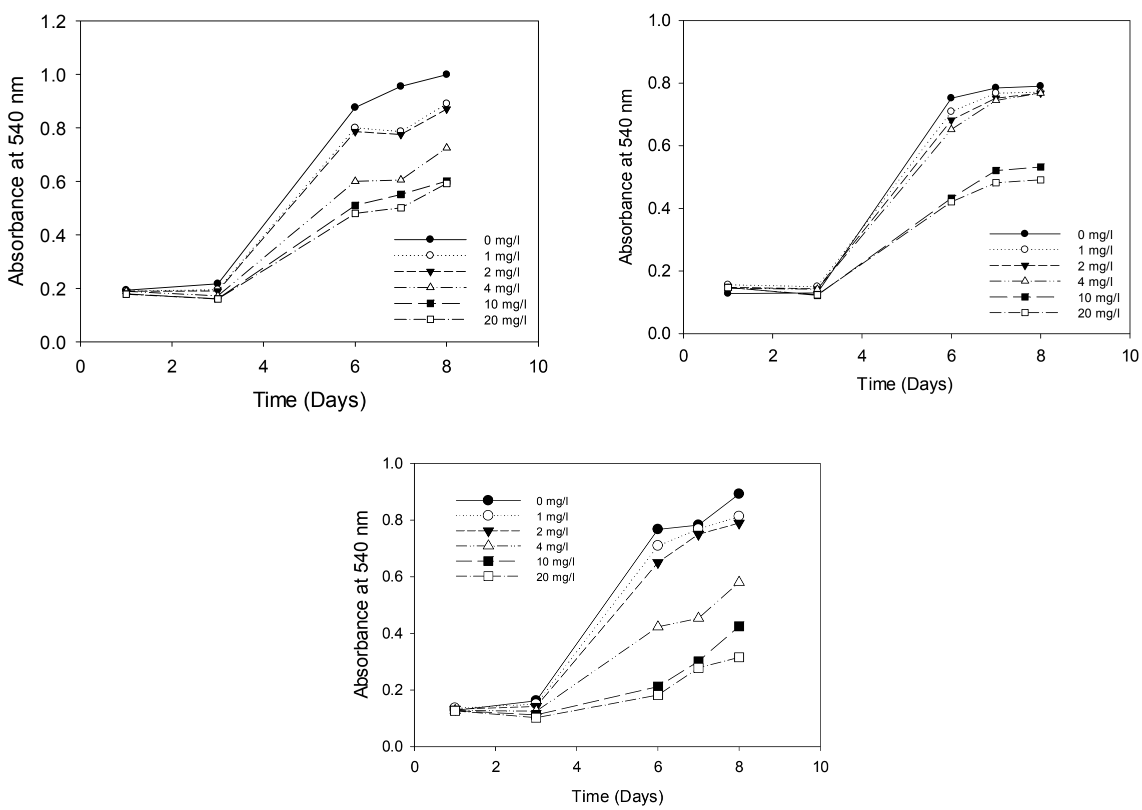


Figure 1: Growth of (a) *Spirulina (Arthospira) platensis*; (b) *Spirulina (Arthospira) indica* and (c) *Spirulina (Arthospira) maxima* in solutions containing aqueous lead (II) ions of various initial concentrations with light intensity of 2000 lux,a 12 h light/dark photoperiod at 30˚C±2˚C, 1: 10 inoculum ratio (0.1gdw *Spirulina*/l), initial solution pH 9 and paddle speed 12 rpm in an open race way pond, was monitored for 8 days.

LCt50 value was found using multiple regression of the lead (II) concentrations and the percentage decrease in productivity values (Table 3: Column 1 and Column 5).

Usually, EC50 values were calculated for biological systems as reported by Chen and Pan (2005), but due to the complexity in applying various formulas and curve fitting of the data, the authors in their previous work (Siva Kiran et al. 2012) simplified the process by applying LCt50 on the *Spirulina* live cells. It is interesting to find that such type of LCt50 values were applied for the first time for *Spirulina sp*. However, the EC50 value represents only the half maximal effective concentration of lead (II) ions (plotted on x-axis) based on 50% response of the adsorption at O.D. 540 nm and it represents only the middle value present in the graph at studied concentrations of metal ions. To find the toxicity, the death of the species based on the metal ion concentrations is required. In the present work, LCt50 was used to predicts the death rate of the species at the exact metal concentration.

Biosorption of lead (II) ions in pilot scale open raceway pond

Raceway ponds are shallow ponds with depth not greater than 50 cm and varied length and breadth based on the required capacity, fitted with paddle wheels for low speed liquid mixing. The medium components are directly exposed to the atmosphere. These types of systems are widely used in commercial scale for cultivation of algae and cyanobacteria like *Spirulina sp, Chlorella sp*, *Anabaena sp* and many others (Jorquera et al. 2010). The present study aims at using open race way pond (0.7 m long, 0.30 m wide, 0.25 m deep) for biosorption of heavy metal ions for purifying waste waters. The working volume is 25 l and detailed conditions were given in culture conditions in materials and methods section. The effect of time on lead (II) biosorption using *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* are shown in Figure 2. The organisms were added separately to open race way ponds containing 1 mg/l of lead (II) ions. The reduction in lead (II) concentration was found at regular intervals up to 1400 min (24 hrs). Maximum biosorption was observed at 7 minutes for *Spirulina (Arthospira) maxima* with 78.8 % of lead (II) and 6 minutes for *Spirulina (Arthospira) platensis* and *Spirulina (Arthospira) indica* with 80.6 % and 74.8 % lead (II) respectively. Further optimization of the process parameters were done at 6 minutes for *Spirulina (Arthospira) platensis* and *Spirulina (Arthospira) indica* and 7 minutes for *Spirulina (Arthospira) maxima*. The maximum adsorption of lead (II) ions after 24 hours for *Spirulina (Arthospira) maxima, Spirulina (Arthospira) platensis* and *Spirulina (Arthospira) indica* are 95 %, 95.8% and 93.9% respectively.

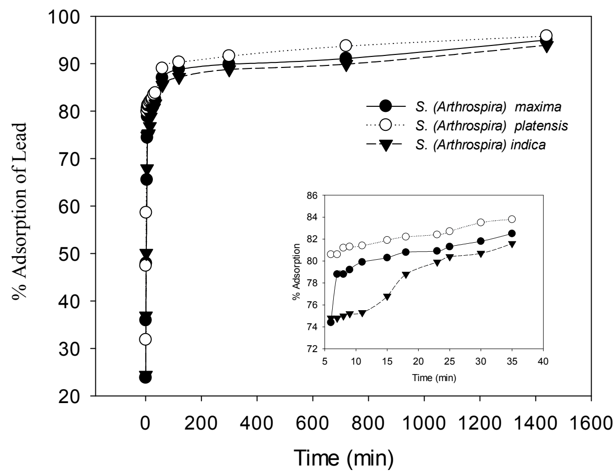


Figure 2: The effect of time on lead (II) biosorption using live *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*.

Table 3: Percentage decrease in the productivity and survival rate of *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica, Spirulina (Arthospira) platensis* on exposure to various lead concentrations in pilot scale open raceway pond.

Lead (II) Concentration	Organism	Percentage decrease in the productivity				LCt50
		1st day	3rd day	7th day	8th day	
1	*Spirulina (Arthospira) platensis*	98.45	89.86	82.30	89.09	24.70 mg/l (7th day)
2		97.92	87.64	81.15	87.13	
4		97.92	79.26	63.39	72.57	
10		92.74	74.19	57.69	60.16	
20		92.22	73.77	52.46	59.25	
1	*Spirulina (Arthospira) maxima*	98.55	92.59	98.20	91.14	6.44 mg/l (7th day)
2		95.65	87.65	95.90	88.45	
4		92.82	77.16	57.92	65.02	
10		92.10	69.13	38.49	47.65	
20		91.37	62.96	35.54	35.33	
1	*Spirulina (Arthospira) indica*	92.85	93.75	97.83	97.60	42.98 mg/l (7th day)
2		88.09	89.5	95.79	97.21	
4		86.30	88.75	94.90	97.19	
10		88.69	76.25	66.36	67.33	
20		87.50	77.50	61.40	62.13	

The adsorption rate was estimated using kinetic parameters based on lead uptake (Figure 2). This will help in selecting the optimal operation conditions for further optimization of batch process. The pseudo first order and pseudo second order equations were applied to the above system for all three species and the kinetic constants were tabulated in Table 4. For solving the first order Lagergren's equation, the equilibrium adsorption capacity q_{eq} was assumed based on the Figure 2 and trial and error method was employed for solving the first order equation and for finding the kinetic constants. In most cases, this first order kinetics does not fit to the entire range of data and it is required to select the data points manually until the equilibrium values for better fit to the model was achieved. A graph is drawn directly for the pseudo second order kinetic equation for the entire range of data points between t/q and t. The slope and intercept were used for finding the kinetic constants (Kalyani et. al. 2009). The Figure 3 clearly states that the second order equation fits better to the above system for all three species when compared with the first order equation confirming the interaction between the functional groups on the cell wall of alga species and the heavy metal ions follows second order kinetics suggested by Ho et al. (1995).

Table 4: Kinetic constants for lead (II) ions biosorption onto live *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*

Organism	Initial Conc. (mg/l)	Pseudo first order kinetics				
		k_1	q_e	R^2	Adjusted R^2	Equation
Spirulina (Arthospira) maxima	1	0.71807	1.9977	0.7426	0.6782	$\log(q_{eq}-q)=0.6920+0.3118t$
Spirulina (Arthospira) indica	1	1.6749	2.7926	0.9028	0.8785	$\log(q_{eq}-q)=1.0270+0.7273t$
Spirulina (Arthospira) platensis	1	1.7122	3.224	0.7784	0.7230	$\log(q_{eq}-q)=1.1706+0.7435t$
		Pseudo second order kinetics				
		k_{II}	q_e	R^2	Adjusted R^2	Equation
Spirulina (Arthospira) maxima	1	0.0612	3.9326	0.9996	0.9996	$t/q=1.0567+0.2543t$
Spirulina (Arthospira) indica	1	0.0743	3.9793	0.9999	0.9999	$t/q=0.8499+0.2513t$
Spirulina (Arthospira) platensis	1	0.0591	3.8865	0.9996	0.9996	$t/q=1.1196+0.2573t$

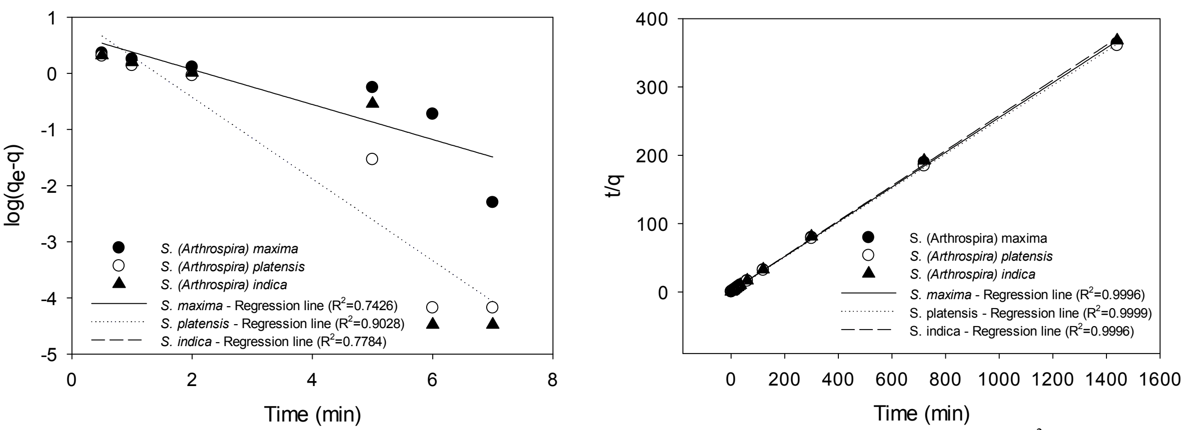


Figure 3: (a) Pseudo first order and (b) Pseudo second order kinetics with regression line and multiple coefficient of regression (R^2) value were shown for *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*

The above results also show that very fast uptake of 74% was observed by the cells before 12 minutes at the beginning as confirmed by Chen and Pan (2005). After 12 minutes, adsorption may take place inside the cells and create gaps on the surface and allowing more molecules to adsorb on the surface (Swift and Forciniti 1997). The kinetic experiments studies by Chojnacka et al. (2005) for biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ion by *Spirulina sp* took less than 5 to 10 minutes for reaching equilibrium. Such rapid adsorption of lead metal ions by *Spirulina sp* is very significant as the death rate of the live *Spirulina* will be very less in 10 minutes. For the second order fit, the difference between adjusted R^2 and R^2 value is found to be very less and near to unity, confirming the significance of the model. For first order system, the R^2 value is far from unity and not closer to the adjusted R^2 showing that more number of the data points does not represent the equation or cannot be fitted into the model. *Spirulina (Arthospira) indica* fits well when compared with the other two species for the second order and first order system based on R^2 value. This may be due to the fact that *Spirulina (Arthospira) indica* has more resistance towards lead when compared with other two species based on the toxicity studies (Table 3).

The effect of initial lead (II) concentration on the biosorbent is shown in Figure 4. The experiments were performed for 7 minutes, 6 minutes and 6 minutes for *S. (Arthrospira) maxima*, *S. (Arthrospira) platensis* and *S. (Arthrospira) indica* respectively in open race way pond with different initial lead (II) concentrations. The percentage adsorption and *Spirulina* gram dry weigh (gdw) were measured and the adsorption capacity q (mg Lead (II)/gdw *Spirulina*) was calculated for all three species. *S. (Arthrospira) indica* showed maximum tolerance at 10 mg/l with 68.5 % lead (II) adsorption, when compared with other two species. The white color symbols represent metal uptake or adsorption capacity q in Figure 4, the maximum metal uptake of 28.2 mg lead (II)/gdw was achieved for *S. (Arthrospira) indica*, 28.25 mg lead (II)/gdw for *S. (Arthrospira) platensis* and 27.25 mg Pb (II)/gdw for *S. (Arthrospira) maxima* at 10 mg/l. *S. (Arthrospira) indica* and *S. (Arthrospira) platensis* showed more tolerance for lead and can be potential adsorbent. The adsorption of the lead was not studied beyond 10 mg/l as it is suggested by Chen and Pan (2005), that at higher lead (II) concentrations, the removal rate decreased due to the saturation of active sites.

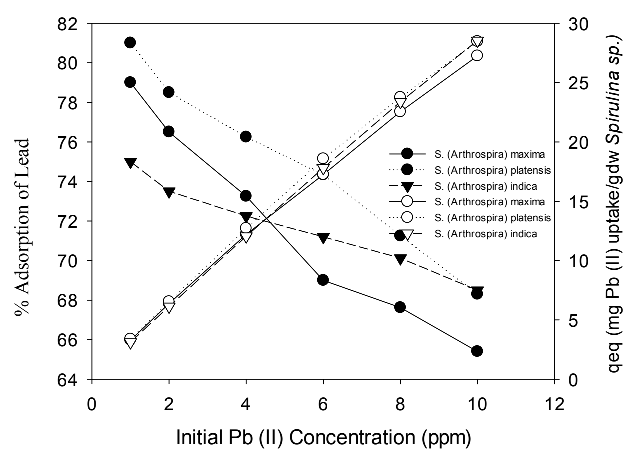


Figure 4: The effect of initial lead concentration on biosorption of *S. (Arthrospira) maxima*, *S. (Arthrospira) platensis and S. (Arthrospira) indica*

To find the relation between the metal uptake and equilibrium

Table 5: Langmuir and Freundlich constants and correlation coefficients for lead (II) biosorption using *S. (Arthrospira) maxima*, *S. (Arthrospira) platensis and S. (Arthrospira) indica*

Organism	Adsorption Isotherm		
S. (Arthrospira) maxima	Langmuir	b	0.2958
	$q_{eq} = \frac{15.4C_{eq}}{1+0.2958C_{eq}}$	q_{max}	52.08
		Correlation coefficient R^2	0.9665
		Adjusted R^2	0.9582
	Freundlich	m	0.7514
	$q_{eq} = 11.0027C_{eq}^{0.7514}$	K_f	11.0027
		Correlation coefficient R^2	0.9984
		Adjusted R^2	0.9979
S. (Arthrospira) platensis	Langmuir	b	0.3137
	$q_{eq} = \frac{17.82C_{eq}}{1+0.3137C_{eq}}$	q_{max}	56.81
		Correlation coefficient R^2	0.9911
		Adjusted R^2	0.9889
	Freundlich	m	0.7698
	$q_{eq} = 12.5545C_{eq}^{0.7698}$	K_f	12.5545
		Correlation coefficient R^2	0.9819
		Adjusted R^2	0.9774
S. (Arthrospira) indica	Langmuir	b	0.1214
	$q_{eq} = \frac{12.51C_{eq}}{1+0.1214C_{eq}}$	q_{max}	103.09
		Correlation coefficient R^2	0.9819
		Adjusted R^2	0.9774
	Freundlich	m	0.8820
	$q_{eq} = 10.7522C_{eq}^{0.8820}$	K_f	10.7522
		Correlation coefficient R^2	0.9993
		Adjusted R^2	0.9992

concentrations, Langmuir and Freundlich models were applied for all three species (*S. (Arthrospira) maxima, S. (Arthrospira) platensis and S. (Arthrospira) indica)*. The adsorption constants, coefficient of determination R^2 and adjusted R^2 along with equations were tabulated (Table 5). For all three organisms, Freundlich empirical model gave better fit when compared with the Langmuir model. There is close relation between R^2 and adjusted R^2 values for Freundlich equation, confirms the significance of the model. Our previous study on cadmium biosorption (Siva Kiran et al. 2012) with *Spirulina (Arthrospira) indica*, also fitted well with Freundlich equation, when compared with Langmuir. Similar results were also obtained by Çelekli and Bozkurt (2011) on the biosorption of nickel and cadmium on *Spirulina platensis*.

Table 6: Experimental plan of the optimization design with the experimental and predicted values for the biosorption of lead (II) ions on open raceway pond using *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica and Spirulina (Arthospira) platensis* as biosorbants.

Run No.	x_1	x_2	x_3	x_4	% Adsorption								
					Spirulina (Arthospira) maxima			*Spirulina (Arthospira) platensis*			*Spirulina (Arthospira) indica*		
					Experiment	Predicted		Experiment	Predicted		Experimental	Predicted	
						BB	ANN		BB	ANN		BB	ANN
1	1	0.1	14	7	77.63	77.951	77.63	79.23	79.556	79.23	73.58	73.359	73.58
2	10	0.1	14	7	64.3	65.024	62.06	69.14	68.758	67.28	66.12	66.352	65.61
3	1	0.2	14	7	80.21	80.579	80.21	82.45	82.846	82.45	76.52	76.214	76.52
4	10	0.2	14	7	68.74	67.653	68.74	70.15	69.824	70.15	69.41	69.574	69.41
5	5	0.15	12	6	66.8	66.081	68.64	72.94	72.775	73.96	69.57	69.273	69.55
6	5	0.15	16	6	65.47	66.046	65.47	73.12	72.746	73.12	70.14	70.960	70.14
7	5	0.15	12	8	64.89	65.378	64.89	72.45	72.831	72.45	69.44	68.554	69.44
8	5	0.15	16	8	65.36	65.343	65.36	73.12	73.292	73.12	71.0	71.231	71
9	5	0.15	14	7	68.8	68.780	68.8	74.22	74.310	74.25	71.32	71.347	71.42
10	1	0.15	14	6	77.9	77.276	77.9	79.52	79.102	79.52	73.2	73.214	73.2
11	10	0.15	14	6	64.1	64.349	64.1	67.18	67.259	67.18	67.43	66.915	67.43
12	1	0.15	14	8	76.9	76.572	76.9	80.01	79.462	80.01	72.9	73.456	72.9
13	10	0.15	14	8	64.2	63.646	64.2	67.51	67.485	67.51	66.19	66.108	66.19
14	5	0.1	12	7	67.4	66.757	67.4	73.85	72.583	73.85	68.54	69.628	68.54
15	5	0.2	12	7	70.21	69.385	70.21	77.98	76.860	77.98	70.88	71.101	70.88
16	5	0.1	16	7	67.12	66.722	67.12	74.11	74.775	74.11	70.5	70.265	70.5
17	5	0.2	16	7	68.74	69.350	69.43	74.29	75.101	72.13	75.93	74.828	76.86
18	5	0.15	14	7	68.8	68.780	68.8	74.28	74.310	74.25	71.52	71.347	71.42
19	1	0.15	12	7	76.23	77.533	76.23	77.65	78.729	77.65	72.3	72.164	72.3
20	10	0.15	12	7	64.21	64.606	64.21	66.21	67.303	66.21	66.42	66.429	66.42
21	1	0.15	16	7	78.54	77.498	78.54	80.21	79.375	80.21	75.22	75.313	75.22
22	10	0.15	16	7	64.3	64.571	64.3	67.42	66.982	67.42	67.21	67.402	67.21
23	5	0.1	14	6	65.69	66.499	65.69	73.62	74.164	73.62	70.05	69.567	70.05
24	5	0.2	14	6	69.42	69.128	69.42	75.32	75.655	75.32	72.3	72.761	72.3
25	5	0.1	14	8	66.61	65.796	66.61	73.54	73.654	73.54	69.9	69.519	69.9
26	5	0.2	14	8	67.20	68.424	67.2	76.86	76.766	76.86	71.8	72.362	71.8
27	5	0.15	14	7	68.74	68.780	68.8	74.43	74.310	74.25	71.2	71.347	71.42

x_1: Initial Concentration (mg/l); x_2: Biosorbant Dosage (gdw/l); x_3: Agitator Speed (rpm); x_4:pH; BB: Box -Behnken;
ANN: Artificial Neural Networks

Table 7: Second order polynomial model for the biosorption of lead onto *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica and* in pilot scale open raceway pond

Organism	Second-order polynomial equation	R^2	Adj. R^2
Spirulina (Arthospira) maxima	$Y = -65.2764 - 3.791x_1 - 104.727x_2 + 7.8x_3 + 24.138x_4 + 0.21x_1^2 + 204.66x_2^2 - 0.3096x_3^2 - 1.829x_4^2 + 2.266x_1x_2 - 0.054x_1x_3 + 0.064x_1x_4 - 2.975x_2x_3 - 15.7x_2x_4 + 0.225x_3x_4$	0.99184	0.98232
Spirulina (Arthospira) platensis	$Y = 9.2838 - 0.669x_1 - 43.18x_2 + 6.9430x_3 + 6.2993x_4 + 0.0132x_1^2 + 533.6667x_2^2 - 0.2036x_3^2 - 0.5846x_4^2 - 2.471x_1x_2 - 0.027x_1x_3 - 0.007x_1x_4 - 9.8750x_2x_3 + 8.1x_2x_4 + 0.0613x_3x_4$	0.98124	0.95935
Spirulina (Arthospira) indica	$Y = 23.492 + 0.53x_1 - 137.157x_2 + 2.107x_3 + 10.930x_4 + 231.333x_2^2 - 0.117x_3^2 - 0.873x_4^2 + 0.41x_1x_2 - 0.06x_1x_3 - 0.06x_1x_4 + 7.725x_2x_3 - 1.75x_2x_4 + 0.124x_3x_4$	0.97063	0.93636

Applications of Box-Behnken experimental design coupled with Artificial Neural Networks in biosorption of lead from three S. (Arthrospira) species.

In the preliminary step of optimization, the selected process variables were fitted to pseudo first order, pseudo second order and adsorption isotherms. The Box-Behnken experimental design is a very useful tool to determine the optimal levels and modeling of process parameters to a second order polynomial equation. The predicted response from the second order polynomial equation, the artificial neural networks and the experimental data of *S. (Arthrospira) maxima*, *S. (Arthrospira) platensis* and *S. (Arthrospira) indica* were tabulated in Table 6. The experiments were done in open race way ponds with culture conditions given in Table 2. Each experiment was repeated three times and the average experimental results were tabulated and repeatability of the experimental results was taken care. The influence of solution pH, initial concentration of lead (II) ions, biosorbent dosage (gdw/l) and agitation speed of the paddle wheel present in the open raceway pond are studied at three levels. The regression equation obtained after application of analysis of variance was also tabulated for all three species (Table 7).

Table 8: Analysis of variance (ANOVA) for the four factorial Box Behnken Experimental design for *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*

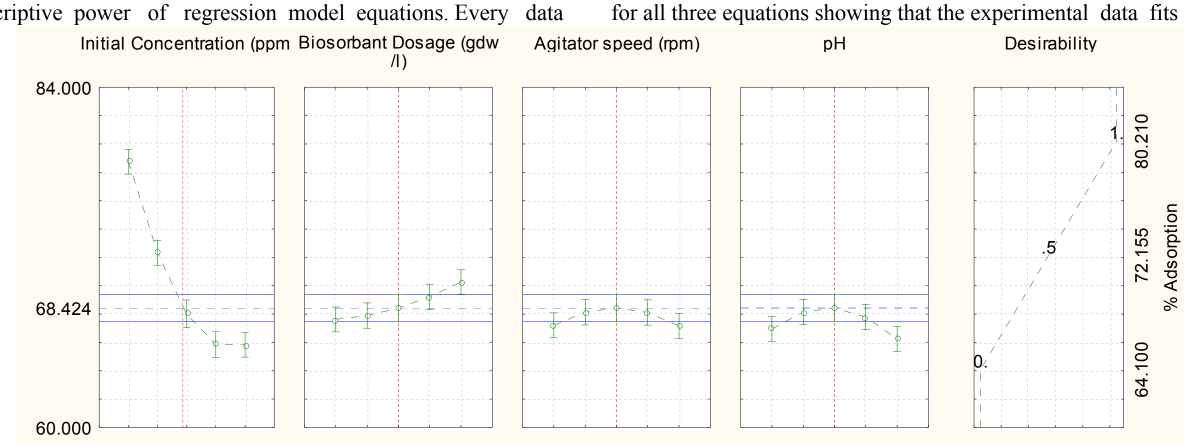
Organism	Source of variation	SS	DF	MS	F-Ratio	p-value
Spirulina (Arthospira) maxima	x_1 & x_1^2	567.0189	2	283.5095	618.9230	0.000000
	x_2 & x_2^2	22.9765	2	11.4882	25.0797	0.000052
	x_3 & x_3^2	8.2120	2	4.1060	8.9637	0.004156
	x_4 & x_4^2	19.1418	2	9.5709	20.8940	0.000123
	$x_1 * x_2$	1.0481	1	1.0481	2.2880	0.156256
	$x_1 * x_3$	0.9386	1	0.9386	2.0490	0.177834
	$x_1 * x_4$	0.3315	1	0.3315	0.7237	0.411571
	$x_2 * x_3$	0.3540	1	0.3540	0.7729	0.396596
	$x_2 * x_4$	2.4649	1	2.4649	5.3811	0.038787
	$x_3 * x_4$	0.8100	1	0.8100	1.7683	0.208317
	Error	5.4968	12	(MSE) 0.4581		
	Total	673.4637	26			
Spirulina (Arthospira) platensis	x_1 & x_1^2	426.3905	2	213.1953	284.3354	0.000000
	x_2 & x_2^2	23.4911	2	11.7455	15.6648	0.000451
	x_3 & x_3^2	3.6169	2	1.8084	2.4119	0.131689
	x_4 & x_4^2	2.0766	2	1.0383	1.3848	0.287658
	$x_1 * x_2$	1.2466	1	1.2466	1.6626	0.221550
	$x_1 * x_3$	0.2358	1	0.2358	0.3145	0.585257
	$x_1 * x_4$	0.0045	1	0.0045	0.0060	0.939316
	$x_2 * x_3$	3.9006	1	3.9006	5.2022	0.041615
	$x_2 * x_4$	0.6561	1	0.6561	0.8750	0.368010
	$x_3 * x_4$	0.0600	1	0.0600	0.0801	0.782045
	Error	8.9976	12	(MSE) 0.7498		
	Total	479.6189	26			
Spirulina (Arthospira) indica	x_1 & x_1^2	141.2894	2	70.64471	143.0075	0.000000
	x_2 & x_2^2	29.0380	2	14.51900	29.3911	0.000024
	x_3 & x_3^2	13.7134	2	6.85670	13.8802	0.000756
	x_4 & x_4^2	4.2989	2	2.14946	4.3512	0.037928
	$x_1 * x_2$	0.0339	1	0.03395	0.0687	0.797660
	$x_1 * x_3$	1.1933	1	1.19326	2.4155	0.146102
	$x_1 * x_4$	0.2771	1	0.27705	0.5608	0.468348
	$x_2 * x_3$	2.3870	1	2.38703	4.8321	0.048292
	$x_2 * x_4$	0.0306	1	0.03063	0.0620	0.807581
	$x_3 * x_4$	0.2450	1	0.24503	0.4960	0.494700
	Error	5.9279	12	(MSE) 0.49399		
	Total	201.8117	26			

DF: Degree of freedom; SS: Sum of squares; MS: mean squares (SS/DF); MSE: Mean Square Error; F: F-Statistics (MS/MSE), 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).

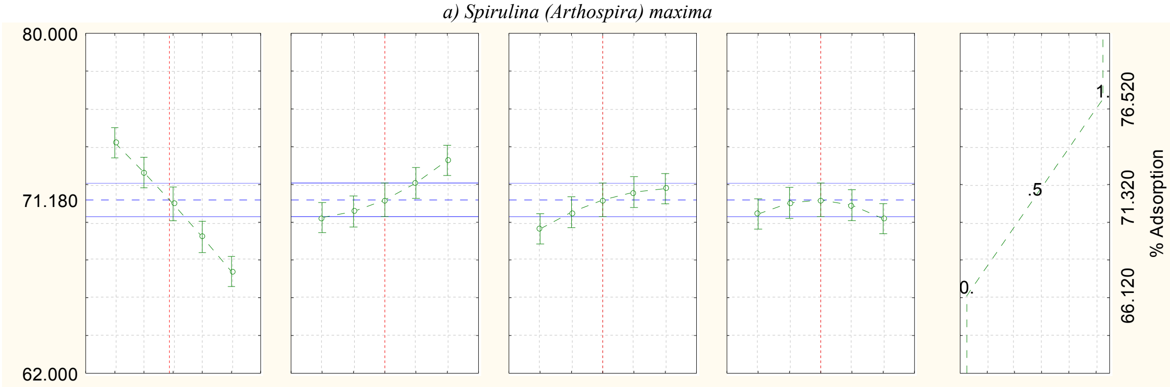
By applying the multiple regression analysis on the above factors and the final adsorption values of all three species, given in Table 6, the following second order polynomial equations were found (Table 7).

Where Y is the predicted lead (II) adsorption percentage and x_1, x_2, x_3 and x_4 are the coded terms for initial concentration (mg/l), biosorbant dosage (gdw/l), agitator speed (rpm) and pH respectively. The difference between R^2 and adjusted R^2, was also tabulated for the above equations for better understanding of the above data. The value of the coefficient of determination ($R^2 = 0.99184$) for *Spirulina (Arthospira) maxima* indicates that only 0.816 (1- R^2) percentage of the total variations in the independent variables (x_1, x_2, x_3 and x_4) were not explained by the model equation. The adjusted R^2 values explain the descriptive power of regression model equations. Every data point added to the model increases the R^2 value for better fit but adjusted R^2 compensates for the addition of variable and it increases and becomes close to the R^2 only if the experimental data points enhances the model and decreases when the data points enhances the model less. Liu et al. (2004) explained about the adjusted R^2, that it corrects the R^2 value for the entire sample size and the number of terms in the model. If there are many terms in the model which does not fits to the equation and the sample size is not very large, adjusted R^2 may be noticeably smaller than the R^2. The value of the adjusted coefficient of determination (Adj R^2 = 0.98232) for *Spirulina (Arthospira) maxima*, are near to the R^2 confirming that that many terms in the model fits the equation.

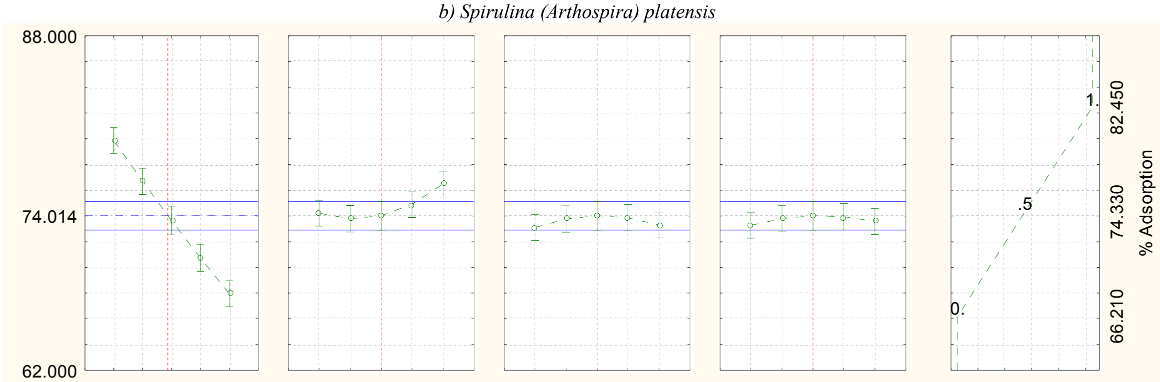
It is clear from the above values, that the R^2 is greater than 0.95 for all three equations showing that the experimental data fits to



a) Spirulina (Arthospira) maxima



b) Spirulina (Arthospira) platensis



c) Spirulina (Arthospira) indica

Figure 5: Profiles for predicted values and desirability showing the individual parametric effects on the adsorption of lead (II) ions on *a) Spirulina (Arthospira) maxima, b) Spirulina (Arthospira) indica* and *c) Spirulina (Arthospira) platensis*.

the predicted equation and the adjusted R^2 value is near to the R^2 for *Spirulina (Arthospira) maxima* and lot of variation was found for other two species. This signifies that most of the experimental data points were fitted significantly to the predicted equation for *Spirulina (Arthospira) maxima* when compared with *Spirulina (Arthospira) platensis* and *Spirulina (Arthospira) indica.* Thus requires better models for fitting the complex experimental data to include those left over data points for better fitness and for better predictability of the second order polynomial equations. The optimal values were obtained by differentiating the above three equations with respect to x_1, x_2, x_3 and x_4 and solving the four equations by equating them to zero. The equations were also analyzed by plotting response surface contour plots and calculating ANOVA (Analysis of Variance - Table 8).

The significance of independent variables and their interactions with each other during the experimentations and for in-depth analysis to find out the most significant process variable which influences the yield were found by ANOVA (Table 8) (Yetilmezsoy et. al. 2009). The p-values and F-values are used as tools to check the significance of each independent variable and also indicates the interaction between them. The smaller the p-value and higher the F-ratio, the more significant the independent variable will become. In the above ANOVA table, initial concentration (mg/l) is found to be more significant when compared with other three independent variables for all three species and next significant factor was found to be live *Spirulina* adsorbant dosage. Agitation speed was found to be more significant for *Spirulina (Arthospira) platensis* and *Spirulina (Arthospira) indica*, when compared to pH. This shows that the selected range of pH is very low (6-8) to show the actual effect on these two species as the solution pH is one of the most important factors of biosorption process, drastically affecting the surface charges on the cell walls of *Spriulina*. Mitrogiannis et al. (2015), showed that biomass of *S (Arthospira) platensis* has optimal initial pH of 7.5 with suitable pH range of 6 to 8. For better understanding of the ANOVA table for the *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*, profiles for predicted values and desirability graphs were plotted (Figure 5) using Statistica V7.0 (Statsoft, USA).

A prediction profile for the initial concentration (mg/l), biosorbant dosage (gdw/l), agitator speed (rpm) and pH for lead (II) ions biosorption on *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* consists of series of graphs of predicted percentage adsorption values for each independent variable and holding the levels of the other independent variables constant. Each individual graph shows the pattern of variation of the percentage adsorption of lead at different levels. Using the above graphs, better understanding of the predicted responses and the most desirable response on the dependent variable can be found (Statistica V7.0, Stafsoft, USA).

If we closely observe the values of initial concentration studied for the Box-Behnken experimental design, (1, 5 and 10 mg/l), the toxicity increases as the metal concentration increases and at high toxicity, the *Spirulina sp.* tends to die leading to the less number of *Spirulina* cells available for biosorption of lead., At low initial concentrations the adsorption percentage will be high and the same pattern was also shown in Figure 4. Chen and Pan (2005), also showed that, as the initial concentration of lead (II) ions increases, the percentage adsorption tends to decreased due to the toxicity and the saturation of the adsorption sites above 20 mg/l. Increase in biosorbent dosage, the percentage adsorption tends to increase and the above graphs clearly shows that when more *Spirulina* was added and more adsorption sites will be available

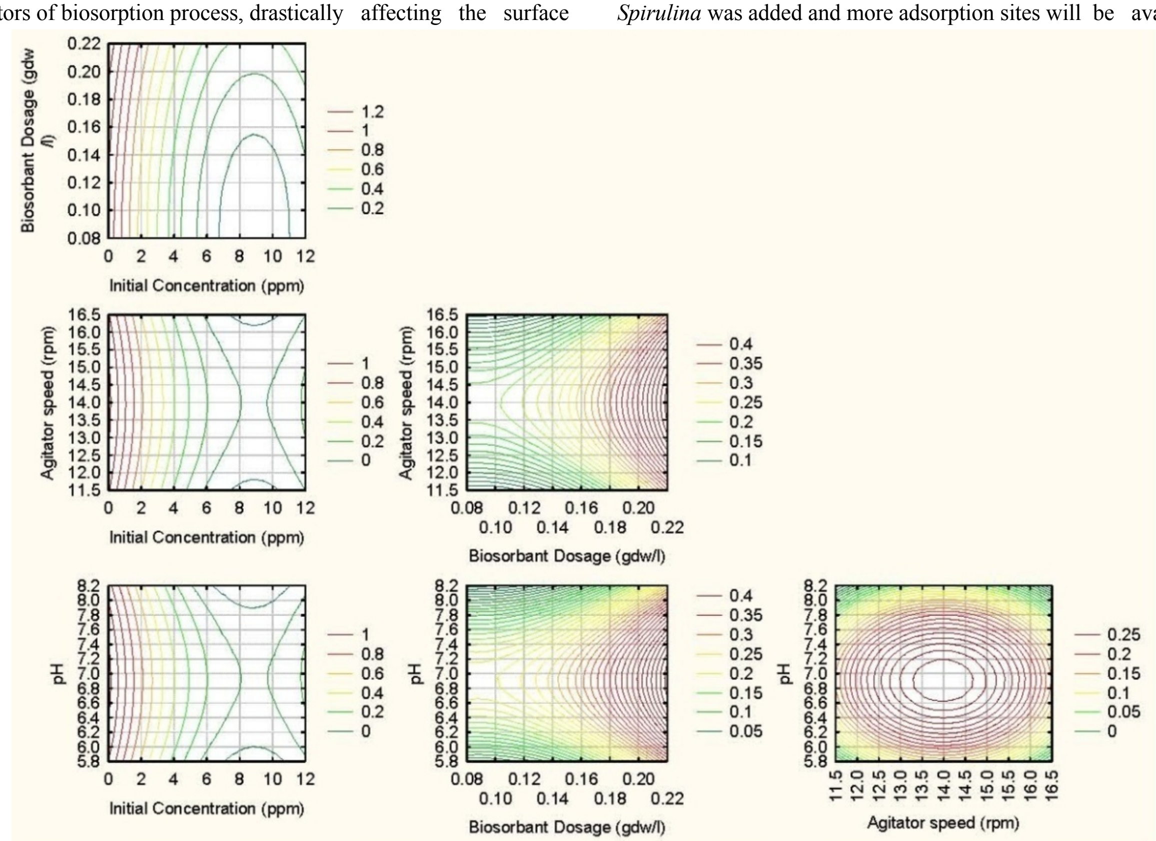


Figure 6: Response surface contour plots for continuous monitoring of the initial concentration (mg/l), biosorbant dosage (gdw/l), agitator speed (rpm) and pH for lead (II) ions biosorption on *Spirulina (Arthospira) maxima*

and more biosorption may take place and the pattern was found to be same for all three *Spirulina sp*. The agitation speed and pH first increased and afterwards decreased for all three species, showing the possibility of getting optimal agitation speed and pH values. Probably at high agitation speeds, more rupture and cell breakage of *Spirulina* might take place leading to the death of many *Spirulina* cells and at low agitation speed, enough contact between the cell walls and the lead (II) ions may be present. Olguin (1994), studied on growth of *Spirulina sp.* at different agitation speeds and found that the variation in growth is unbalanced and not following any pattern. Optimal pH was found to be about 7 and it has to be further verified by solving the above predicted equations tabulated in Table 7.

The two dimensional contour plots were drawn to determine the interactions of the initial concentration, biosorbant dosage, agitator speed and pH on adsorption of lead (II) ions on to *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis*. The Figures 6, Figure 7 and Figure 8, shows the relative interaction of any two factors when the other two factors were maintained at constant level. In Figure 6, pH and agitation speed showed correct pattern by giving optimal values at pH 7 and agitation speed 14 rpm, the remaining graphs are considered to be having saddle points i.e. they are having curves up in one direction and curves down in other direction and does not have optimal points. The circle represents optima minima or optima maxima and the trend towards reaching

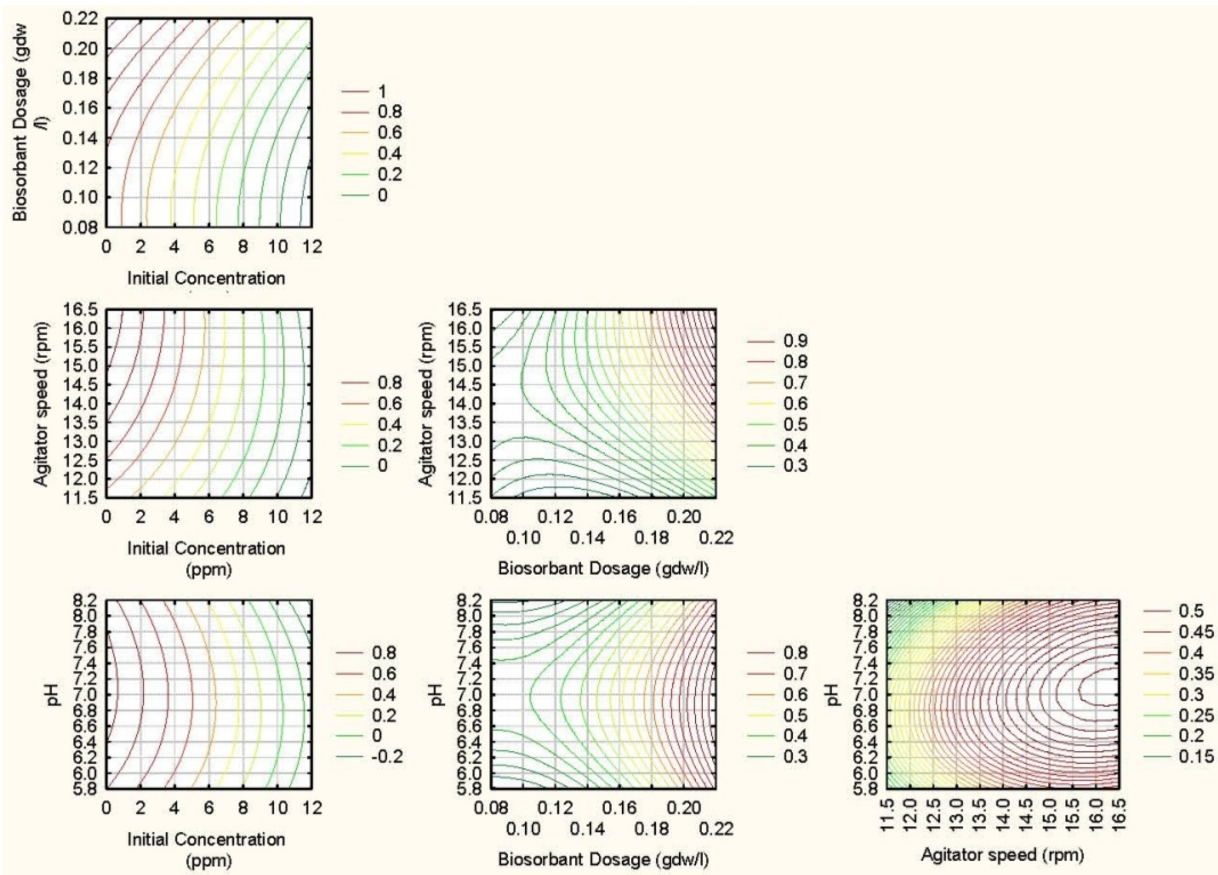


Figure 7: Response surface contour plots for continuous monitoring of the initial concentration (mg/l), biosorbant dosage (gdw/l), agitator Speed (rpm) and pH for lead (II) ions biosorption on *Spirulina (Arthospira) indica*

Derringer and Suich (1980) developed a procedure for specifying the relationship between predicted responses (percentage biosorption) on a dependent variable and the desirability of the responses which is called as the desirability function. He also formulated a procedure that provides three "inflection" points in the function represented in the graph. The score “0” can tend to be less desirable and score “1” is highly desirable and score “0.5” can be in between factor. The “higher is better”, desirability function was used for plotting the above graph. As the graph clearly shows that at lowest initial concentrations, the highest desirability score was shown for all three species. The average range is found to be from 60 % to 83 % for all experiments (desirability values) shown in Figure 5. Response surface contour plots were plotted for finding the optimal values and understanding the interactive responses between each factor (Figure 6, Figure 7 and Figure 8).

the circle, represents that the system is reaching towards the optimal adsorption rate. Here in the above graphs, optima maxima was achieved. The graphs, between pH and biosorbant dosage and agitation speed and biosorbant dosage, the optimal pH is approximately near to 6.6 and 7.4 and optimal agitation speed is at approximately between 13.5 rpm and 14.5 rpm but the both graph tends to move towards highest biosorbent dosage. At 0.12 gdw/l biosorbent dosage the percentage removal of lead (II) ions is low and at 0.22 gdw/l, the biosorbent dosage is high and the graph trend is moving towards higher biosorbent dosage for higher percentage adsorption. These graphs will help in in-depth analysis of the variation of the independent variables.

Figure 7 shows maximum biosorption was found to be near pH 6.8 and 7.4 and agitation speed beyond 16 rpm. The remaining graphs found to be more saddle points with curve patterns at different directions leading to no specific conclusion. But graphs including initial concentration shows that at low initial concentrations, the percentage adsorption is high and at high biosorbent dosage i.e. >22 gdw/l, the adsorption percentage is found to be high. The graph trend seems to be complicated and may affect the prediction of optimal points.

like training function, transfer function, error, training set selection or construction of topology for the neural networks. The choice of the design parameters for the neural networks was determined by trial and error method. In present study, the testing experimental run data set and along with other parameters were

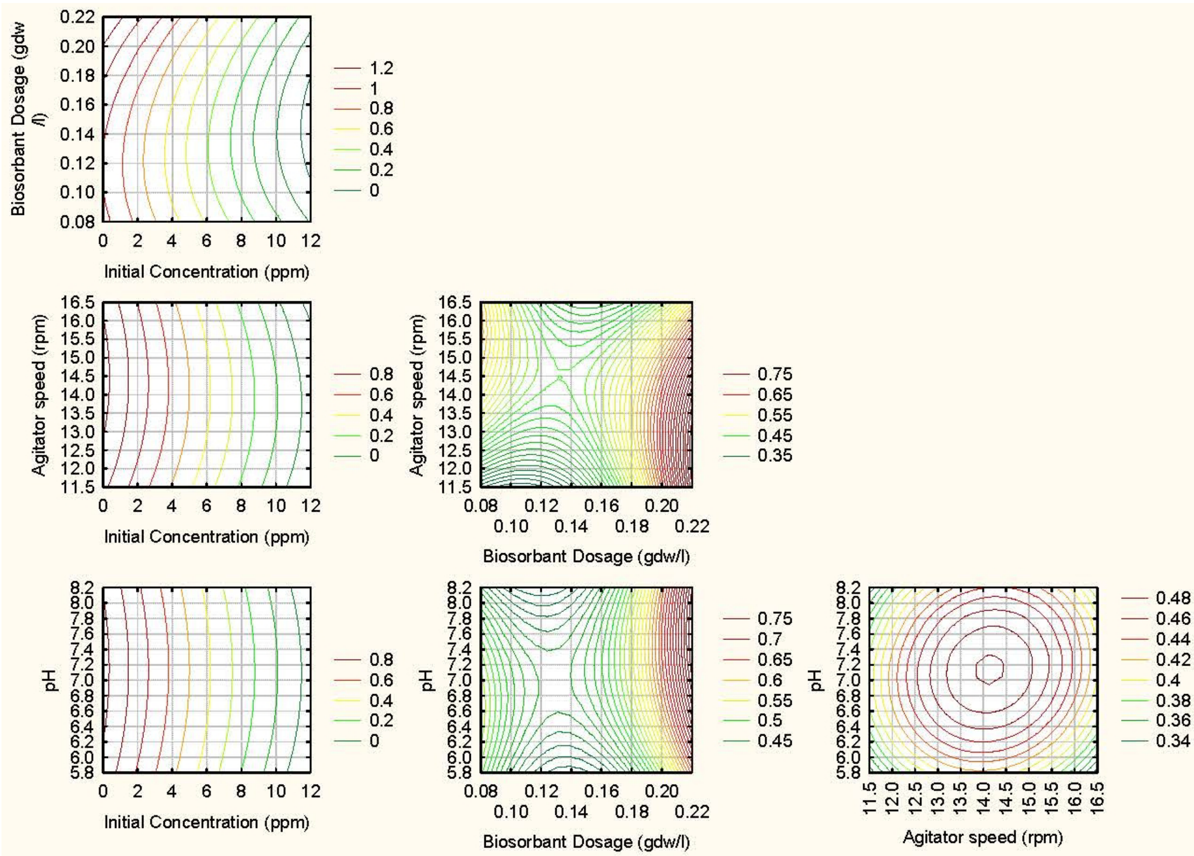


Figure 8: Response surface contour plots for continuous monitoring of the initial concentration (mg/l), biosorbant dosage (gdw/l), agitator Speed (rpm) and pH for lead (II) ions biosorption on *Spirulina (Arthospira) platensis*

For *Spirulina (Arthospira) platensis* the optimal pH, lies in the range of 7.0 to 7.4 and optimal agitation speed (rpm) in the range of 13.5 rpm to 14.5 rpm and is approximately similar to the other two species. The other graphs showed saddle points with one or more optimal points but following the same pattern that at low concentrations the yield is high and at high biosorbent dosage the removal rate is less. Table 10 showed predicted responses at optimal Initial concentration, biosorbant dosage, agitator speed and pH of the all three species. The values from the Box-Benkehn results failed to predict the exact parameters. Some initial concentration values were shown negative and some beyond 500 mg/l which were absolutely wrong and the predicted percentage also goes beyond 100 %. The same saddle pattern was also shown in the above contour graphs and still more advanced stochastic models are required to predict the optimal points from the Box-Behnken Experimental design data.

The input and hidden layers included for the above experimental data and network topology used are given in Table 9 along with the back propagation training function. For testing, the run numbers, 2, 5, 17 and 27 were used for all three species and the remaining experimental runs were used for training. As per Nagata and Chu (2003), there are no specific guidelines exist for the selection of any of the above mentioned design parameters selected after running various trials and comparing the results for better Coefficient of Determination (R^2) value.

The configuration of the neural network developed in this work (Eg: 4-24-1 structure: four input neurons - twenty four neurons in hidden layer - one output neuron - *Spirulina (Arthospira) maxima* - Table 9 - Column 2) was determined by trial and error method. Because of the empirical nature of the neural networks, the data sometimes will not extrapolate well and final experimentation is also required to prove the validity of the predicted model.

Ravi et al (2012), suggested an equation for neural network training for calculation of weights and bias by the artificial neural networks. A non linear mapping between the input parameters, for the experimental runs was determined based on the equation suggested by Ravi et. al. (2012). The equation contains the input parameters (x_1, x_2, x_3 and x_4), output parameters (y) and weights and the bias suggested by the neural networks based on the training using training function "trainlm".

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

Where w_1 and w_2 are the weights, b_1 and b_2 are the biases (Table 9). '**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 (4x1). A matlab code was written using "postreg" function to test the training data with testing data and for finding the coefficient of regression (R^2). , the parameters like total number of neurons, testing set, mean square error, various training functions available in Matlab Software (*trainbr*, *trainb* etc..), number of iterations were changed until maximum R^2 was achieved. After considerable number of trials, the testing set, training set, training function was finalized and the optimal numbers of hidden neurons were found. The theoretical and calculated weights and bias for all three species was optimized using DIRECT algorithm suggested by Jones et al. (1993).

Table 9: Weights and biases of the neural networks for modeling the Box-Behnken experimental design data for biosorption of lead (II) ions on live *Spirulina (Arthospira) maxima, Spirulina (Arthospira) indica* and *Spirulina (Arthospira) platensis* in open race way pond

Organism	Training function	Weights and biases of the neural networks						
		w1				w2	b1	b2
Spirulina (Arthospira) maxima	Levenberg -Marquadt (trainlm) $R^2 = 0.99365$ 24 Neurons (4-24-1)	-0.4023	-0.8513	-0.9613	-0.5166	-8.49949	-6.0254	19.3431
		-4.4069	-0.9581	3.9656	-0.6618	1.355852	-1.3374	
		3.0092	-1.1843	1.9048	-2.2567	-1.05383	1.3157	
		2.1115	0.7533	-0.5915	1.4802	4.36521	7.9427	
		1.3216	0.1163	-0.7143	-1.427	-4.56108	-7.8747	
		0.9834	0.83	-0.8622	-1.3878	3.091108	6.2305	
		0.6892	-1.1841	0.2713	-0.2223	-1.02376	-4.3593	
		-0.2066	-3.2524	0.0686	-0.5282	1.513502	-6.0771	
		2.1369	-2.296	0.3598	2.5792	-3.06022	-0.6159	
		5.709	1.9422	-0.4444	0.5041	1.642421	-2.705	
		4.8739	0.8126	0.9253	-0.7382	-3.0343	1.5747	
		-2.6385	1.9821	4.2369	0.058	0.594338	-0.0469	
		1.7695	2.4045	3.0908	-2.6437	-0.42172	-0.44	
		0.077	-0.1044	-0.6816	0.0105	-3.12884	-3.4064	
		3.1543	0.6372	0.1671	3.0667	-2.11481	-0.298	
		-1.0004	-2.2385	-1.2329	-1.3599	1.819952	5.6418	
		-0.4481	-1.9552	-2.9831	-0.3479	-0.63597	-2.5759	
		0.1899	1.3917	-1.7704	2.4433	4.969359	3.9644	
		0.1297	1.0227	-0.2433	-0.9508	0.114859	4.8713	
		-2.4154	1.7099	0.4518	-1.0939	1.902598	6.3779	
		-4.9597	2.3579	-1.5192	4.6358	3.657721	7.9318	
		1.7476	1.3149	2.4148	2.4294	-6.71012	-6.8073	
		-8.1011	1.8651	-2.9016	-2.1645	-1.3302	2.6285	
		-0.6502	0.0804	1.111	-1.1358	9.850148	5.1247	
Spirulina (Arthospira) platensis	Levenberg -Marquadt (trainlm) $R^2 = 0.99133$ 16 Neurons (4-16-1)	-0.0155	-1.4025	0.4956	1.1872	-13.5375	-3.7446	32.3216
		3.0142	-8.4843	5.1057	5.8793	3.0359	-3.3421	
		-0.6657	-0.3903	-0.9053	0.7541	5.639	3.7168	
		-2.5438	6.6103	7.3067	-6.8031	2.0296	6.8958	
		1.4274	0.4812	1.8743	1.0842	-7.5783	-2.5739	
		-0.2137	-1.4548	-0.0954	0.6175	2.7795	3.8837	
		0.2724	-4.1507	-4.9706	4.5502	-2.4707	-1.5138	
		1.7752	1.063	-0.5782	-2.0862	-4.8859	-4.529	
		-0.8381	3.4265	1.2659	0.6536	0.7303	0.7223	
		-3.7264	-0.9188	-1.8383	0.5934	2.7487	-2.0942	
		6.8246	0.881	5.4059	3.2231	-3.3854	-2.6885	
		2.7314	-3.144	-0.1441	-2.0888	-1.8834	1.5049	
		-3.6704	0.932	-2.2885	0.3539	2.5188	8.1595	
		-5.7881	-4.1583	-2.9772	-2.875	-2.9315	3.0827	
		0.7246	-0.1939	0.5853	-1.4286	-9.323	-4.4534	
		7.6032	-9.5617	-0.0415	7.7447	2.3029	-8.4334	
Spirulina (Arthospira) indica	Levenberg -Marquadt (trainlm) $R^2 = 0.9979$ 17 Neurons (4-17-1)	-1.2104	-1.6347	-1.9083	2.7642	9.6041	2.5878	36.6874
		-7.6862	-2.8572	-2.7228	5.297	1.704	-3.978	
		-1.6642	-0.0331	5.5177	-0.6777	9.0509	11.8496	
		-0.6631	4.0782	0.2409	-0.1494	4.1176	9.6424	
		-0.918	1.2658	0.5173	1.0422	1.0796	4.2038	
		0.854	-0.7178	0.0142	-1.9428	0.0631	-1.0814	
		0.1877	-4.2794	-1.7238	1.053	-1.0696	2.1991	
		0.0992	-0.9447	-1.3344	4.1519	-0.5563	0.5698	
		-2.7054	-0.5269	0.631	-0.0293	3.3779	1.5036	
		-1.7581	-2.5563	-6.2118	-2.2055	-2.4377	-6.0178	
		1.7577	1.5734	0.3791	2.3608	-2.6487	-2.851	
		-1.0622	5.1582	2.3588	2.6083	-1.6445	0.5761	
		-2.5824	-0.5376	0.1074	-6.2088	1.7371	3.8388	
		-1.8462	5.1077	-0.7472	0.0858	2.0359	1.1326	
		-1.4301	-0.6751	-0.0834	-2.3528	5.1295	3.506	
		-3.9038	-2.9297	-0.6901	0.7272	7.8448	9.0402	
		-9.5101	-6.27	-5.9499	0.5251	-9.7264	4.7664	

The optimal values predicted by Box-Behnken experimental design by solving four equations arising by differentiating the response variable of the equations given in Table 7 for all three species and artificial neural networks using DIRECT algorithm were tabulated in Table 10. The initial concentration for *Spirulina (Arthospira) platensis* and negative concentration for *Spirulina (Arthospira) indica* are not acceptable. .The fact that Box-Behnken failed to predict lies in the profile prediction graphs of lead (II) concentration and high for biosorbent dosage. Our interest is to find the optimal maximum lead (II) removal within the confined region of experimentation levels. 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 6) for all three species. This demonstrates the superiority of the ANN and the DIRECT algorithm when compared with

Table 10: Optimized values predicted by global optimization algorithm linked to artificial neural networks

Organism Name		Optimized values based on the model equation					
		Initial Concentration (mg/l)	Biosorbant Dosage (gdw/l)	Biosorbant Dosage (gdw/l)	pH	% Adsorption	
						Predicted	Experimental
Spirulina (Arthospira) maxima	Box-Benkhen Experimental Design	88.96652	0.08579	13.98587	6.90389	64.74	-
Spirulina (Arthospira) platensis	Box-Benkhen Experimental Design	557.5316	0.1288	14.1217	7.176	40.167	-
Spirulina (Arthospira) indica	Box-Benkhen Experimental Design	-387.475	0.085	16.282	6.930	87.3617	-
Spirulina (Arthospira) maxima	Artificial Neural Networks	10.1852	0.164815	13.9342	7.14815	80.7175	79.32
Spirulina (Arthospira) platensis	Artificial Neural Networks	10.0617	0.138477	15.284	7.53498	88.1518	83.58
Spirulina (Arthospira) indica	Artificial Neural Networks	48.3333	0.177984	15.6461	7.37037	90.2168	91.15

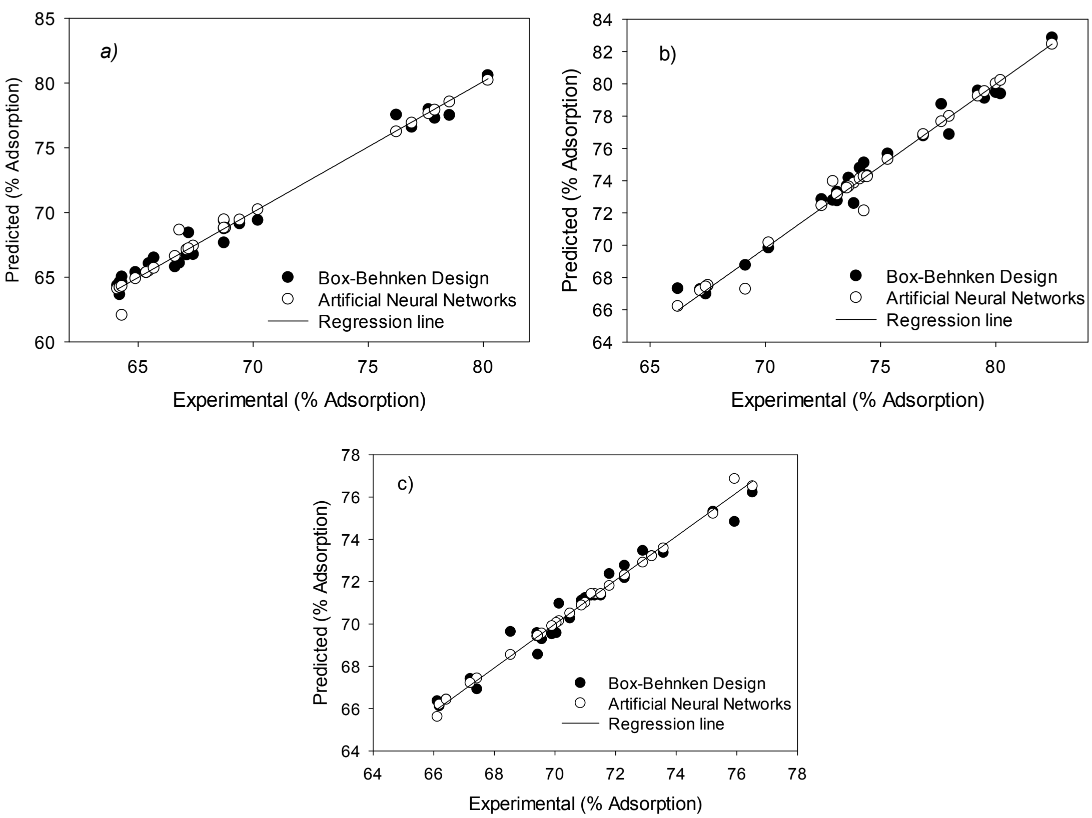


Figure 9: The three graphs shows, the predicted percentage adsorption from second order polynomial equation from Box-Behnken experimental design, equation from weights and bias from artificial neural networks, and the regression line with the experimental percentage adsorption for *a) Spirulina (Arthospira) maxima, b) Spirulina (Arthospira) indica* and c) *Spirulina (Arthospira) platensis*.

initial lead (II) concentration and biosorbent dosage is shown in the Figure 5. When concentration decreases, the adsorption increases and when biosorbant dosage increases, then adsorption percentage increases. If the data is blindly extrapolated or interpolated without applying non linear stochastic intelligent algorithms, the predicted values will become negative for initial Box-Behnken experimental design. The Box-Behnken predicted values were neglected as it is impossible to maintain negative predicted concentrations and near zero biosorbant dosage. The neural network model not only fits the training data very well but also provides predictions of the validation data very close to those measured experimentally (Nagata and Chu 2003). The open circles in the above diagram (Figure 9), which are little far to the regression line are the predicted responses from the ANN and are excluded from the actual training.

Conclusions

In this present work, the importance of using live *Spirulina sp.* for biosorption of heavy metal ions and the importance and possibility of using algal open raceway ponds for waste water treatment was explored. The growth and toxicity studies of live cells of *S. (Arthrospira) maxima, S. (Arthrospira) indica* and *S. (Arthrospira) platensis* in algal open raceway ponds were done. *Spirulina (Arthrospira) indica* is more tolerant when compared with other two species. The LCt50 values for all three species were estimated. The same algal open raceway pond were used for biosorption of lead using live *Spirulina sp.* and models like, Lagergren's first order equation, pseudo second order equation and Langmuir and Freundlich adsorption isotherms were fitted to the adsorption experimental data. Empirical model building like second order polynomial equation was a standard approach for handling statistical experimental designs like Box-Behnken was used for the present study, it is found that neural networks provided better fit when compared with conventional quadratic model. The DIRECT algorithm is successfully applied for the first time in finding the optimal values for complex biosorption systems using *Spirulina sp.* This study establishes the possibility of using open race way ponds for wastewater treatments and superiority of using ANN systems coupled with DIRECT algorithm for handling complex adsorption multi-factorial systems.

Acknowledgements

The authors would like to thank the management of M. S. Ramaiah Institute of Technology for providing laboratory facility and special industrial training on *Spirulina* at Nallayan Research Centre, Navalur, Chennai, Tamil Nadu. The authors wish to thank Dr. G. Hanumantha Rao, Professor, Anil Neerukonda Institute of Technology and Sciences (ANITS), Visakhapatnam, Andhra Pradesh for helping in mathematical modeling and artificial neural networks.

Reference

Anirudhan TS, Ramachandran M (2015) Adsorptive removal of basic dyes from aqueous solutions by surfactant modified bentonite clay (organoclay): Kinetic and competitive adsorption isotherm. Process Safety and Env Prot 95:215-225

Annadurai G, Sheeja RY (1998) Use of Box-Behnken design of experiments for the adsorption of verofix red using biopolymer. Bioprocess Eng 18(6):463-466

Babu P Nirguna, Prakash Binnal, D. Jayaprasanna Kumar (2015) Biosorption of Zn+ 2 on non living biomass of *Spirulina platensis* immobilized on polyurethane foam cubes: Column studies. J Biochem Tech 6(1):852-859

Bailey, Norman TJ (1995) Statistical methods in biology. Cambridge University Press, USA.

Balaji S, Kalaivani T, Rajasekaran C (2014) Biosorption of Zinc and Nickel and Its Effect on Growth of Different *Spirulina* Strains. CLEAN–Soil, Air, Water 42(4):507-512

Box, George EP, Donald W. Behnken (1960) Some new three level designs for the study of quantitative variables. Technometrics 2(4):455-475

Çelekli A, Bozkurt H (2011) Bio-sorption of cadmium and nickel ions using *Spirulina platensis*: Kinetic and equilibrium studies. Desalination 275(1):141-147

Chen H, Pan SS (2005) Bioremediation potential of spirulina: toxicity and biosorption studies of lead. J Zhejiang Uni Sci B 6(3):171

Cheng Y, Yang C, He H et al (2015) Biosorption of Pb (II) Ions from Aqueous Solutions by Waste Biomass from Biotrickling Filters: Kinetics, Isotherms, and Thermodynamics. J Env Eng. doi:10.1061/(ASCE)EE.1943-7870.0000956

Chiter L (2006) DIRECT algorithm: A new definition of potentially optimal hyperrectangles. App Math Comp 179(2):742-749

Chojnacka K, Chojnacki A, Gorecka H (2005) Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae *Spirulina sp.:* kinetics, equilibrium and the mechanism of the process. Chemosphere 59(1):75-84

Crist RH, Oberholser K, Shank N, Nguyen M (1981) Nature of bonding between metallic ions and algal cell walls. Env Sci Tech 15(10):1212-1217

Jones DR, Perttunen CD, BE Stuckman (1993) Lipschitzian Optimization Without the Lipschitz Constant, JOTA 79(1)

Derringer G, Suich R (1980) Simultaneous optimization of several response variables. J Qual Tech 12:214-219

Deniz F, Kepekci RA (2015) Equilibrium and kinetic studies of azo dye molecules biosorption on phycocyanin-extracted residual biomass of microalga Spirulina platensis. Desalination Water Treatment (ahead-of-print) 1-7

Dole, Marjorie Welsh (1952) The *Aspergillus niger* method for determining copper in soils. Soil Sci 73(2):135-148

Domozych David S (2011) Algal cell walls, John and Wiley Publications, USA

Dotto GL, Lima EC, Pinto LAA (2012) Biosorption of food dyes onto Spirulina platensis nanoparticles: equilibrium isotherm and thermodynamic analysis. Biores tech 103(1):123-130

Freundlich HMF (1906) Über die Adsorption in Lösungen. Z Phys Chem 57(A):385–470

Gong R, Ding Y, Liu H et al. (2005) Lead biosorption and desorption by intact and pretreated Spirulina maxima biomass. Chemosphere 58(1):125-130

Liu HL, YW Lan, YC Cheng (2004) Optimal production of sulphuric acid by *Thiobacillus thiooxidans* using response surface methodology, Process Biochem. 39:1953–1961

Harris John O, A Eisenstark et al (1954) A study of the location of adsorbed mercuric ions in *Escherichia coli*. J Bact 68(6):745

Hegde Shubha M, RL Babu, E Vijayalakshmi et al (2015) Biosorption of hexavalent chromium from aqueous solution using chemically modified Spirulina platensis algal biomass: an ecofriendly approach. Desalination and Water Treat (ahead-of-print):1-10

Ho YS (1995) Absorption of heavy metals from waste streams by peat (Doctoral dissertation, University of Birmingham).

Ho YS, Mckay G (1998) A comparison of chemisorption kinetic models applied to pollutant removal on various sorbents, Process Safety and Env Protection 76B:332.340

Hu YS (2004) Citation review of Lagergren kinetic rate equation on adsorption reactions. Scientometrics 59(1):171-177

FAO (2010) Food and Agriculture Organization of the United Nations Report on "World Review of Fisheries And Aquaculture", (http://www.fao.org/docrep/013/i1820e/i1820e01.pdf

Hu X, Li Y, Chen Y (2015) A direct algorithm of one-dimensional optimal system for the group invariant solutions. J Mat Phy 56(5):1-12

Jones Donald R, Cary D Perttunen, Bruce E Stuckman (1993) Lipschitzian optimization without the Lipschitz constant. J Opt Theory Applications 79(1):157-181

Jorquera O, Kiperstok A, Sales EA et al (2010) Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. Bioresource Tech 101(4):1406-1413

Kaan Y, Sevgi D (2008) Artificial neural network (ANN) approach for modeling of Pb(II) adsorption from aqueous solution by Antep pistachio (Pistacia Vera L.) shells. J Hazard Mater 153:1288-1300.

Kalyani G, Babu Rao G, Vijaya BS, Prasanna, YK (2009) Equilibrium and kinetic studies on biosorption of zinc onto *Gallus domesticus* shell powder. ARPN J Eng App Sci 4(1):39-49

Kwak HW, Kim MK, Lee JY et al (2015) Preparation of bead-type biosorbent from water-soluble *Spirulina platensis* extracts for chromium (VI) removal. Algal Res 7:92-99

Lagergren, Svenska (1898) About the theory of so-called adsorption of soluble substances. Kungliga Svenska Vetenskapsakademiens Handlingar 24(4):1-39

Langmuir I (1918) The adsorption of gases on plane surfaces of glass, mica and platinum. J American Chem Society 40(9):1361-1403

Limousin G, Gaudet JP, Charlet L et al (2007) Sorption isotherms: a review on physical bases, modeling and measurement. App Geochem 22(2):249-275

Markou G, Mitrogiannis D, Çelekli A et al (2015) Biosorption of Cu^{2+} and Ni^{2+} by *Arthrospira platensis* with different biochemical compositions. Chem Eng Journal 259:806-813

Mitrogiannis D, Markou G, Çelekli A et al (2015) Biosorption of methylene blue onto *Arthrospira platensis* biomass: Kinetic, equilibrium and thermodynamic studies. J Env Chem Eng 3(2):670-680

Moré Jorge J (1978) The Levenberg-Marquardt algorithm: implementation and theory." Numerical analysis. Springer Berlin Heidelberg, Germany

Nagata Y, Chu KH (2003) Optimization of a fermentation medium using neural networks and genetic algorithms. Biotech letters 25(21):1837-1842

Olguin EJ, B Hernández, A Araus et al (1994) Simultaneous high-biomass protein production and nutrient removal using *Spirulina maxima* in sea water supplemented with anaerobic effluents. World J Microbiol biotech 10(5):576-578

Peters RW, Shem L (1993) Separation of heavy metals: removal from industrial wastewaters and contaminated soil. Argonne National Lab, IL (United States)

Prakash, GVS Bhanu, V. Padmaja, RR Siva Kiran (2008) Statistical optimization of process variables for the large-scale production of *Metarhizium anisopliae* conidiospores in solid-state fermentation." Bioresource Technology 99(6):1530-1537

Puranik PR, KM Paknikar (1997) Biosorption of lead and zinc from solutions using *Streptoverticillium cinnamoneum* waste biomass. J biotech 55(2):113-124

Puranik PR, KM. Paknikar (1999) Biosorption of lead, cadmium, and zinc by *Citrobacter* strain MCM B ‐ 181: Characterization Studies. Biotechnology progress 15(2):228-237

Rangsayatorn N, Upatham ES, Kruatrachue M et al. (2002) Phytoremediation potential of *Spirulina (Arthrospira) platensis*: biosorption and toxicity studies of cadmium. Env Pol 119(1):45-53

Ravi Kumar Dasari VR, Reddy Donthireddy SR, Yugandhar Nikku M, Rao Garapati H (2009) Optimization of medium constituents for Cephalosporin C production using response surface methodology and artificial neural networks. J Biochem Tech 1(3):69-74

Rogers Jonathan N, Julian N Rosenberg, Bernardo J Guzman et al (2014) A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. Algal Res 4:76-88

Seker A, Shahwan T, Eroğlu AE et al. (2008) Equilibrium, thermodynamic and kinetic studies for the biosorption of aqueous lead (II), cadmium (II) and nickel (II) ions on *Spirulina platensis*. J Hazard Mat 154(1):973-980

Siva Kiran RR, Konduri R, Rao GH, Madhu GM (2010) Statistical optimization of endo-polygalacturonase production by overproducing mutants of *Aspergillus niger* in solid-state fermentation. J Biochem Tech 2(2):154-157

Siva Kiran RR, Madhu GM, Satyanarayana SV, Bindiya P (2012) Bioaccumulation of cadmium in blue green algae *Spirulina (Arthrospira) indica*. J Bioremediation Biodegradation 3(3):1-4

Solisio C, Lodi A, Torre P et al. (2006). Copper removal by dry and re-hydrated biomass of Spirulina platensis. Bioresource tech 97(14):1756-1760

Swift DT, Forciniti D (1997) Accumulation of lead by Anabaena cylindrica: mathematical modeling and an energy dispersive X-ray study. Biotech Bioengg 55:408-419.

Turan NG, Mesci B, Ozgonenel O (2011) The use of Artificial Neural Networks (ANN) for modeling of adsorption of Cu (II) from industrial leachate by pumice. Chem Eng J 171(3):1091-1097

World Health Organization (2011) Lead in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality, WHO, Geneva, Switzerland

Yetilmezsoy K, Demirel S (2008) Artificial neural network (ANN) approach for modeling of Pb (II) adsorption from aqueous solution by *Antep pistachio* (*Pistacia Vera L.*) shells. J Haz Mat 153(3):1288-1300

Yetilmezsoy K, Demirel S, Vanderbei RJ (2009) Response surface modeling of Pb (II) removal from aqueous solution by *Pistacia vera* L.: Box–Behnken experimental design. J Haz Mat 171(1):551-562

Zarrouk C (1966) Influence de divers facteurs physiques et chimiques sur la croissance et photosynthese de *Spirulina maxima*, University of Paris, France