ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Optimization using central composite design (CCD) for the biosorption of Cr (VI) ions by *Azolla filiculoidus* a fresh water macro alga.

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ABSTRACT

The presence of Chromium in aqueous streams arising from the discharge of industrial effluents into water bodies is one of the most important environmental issues because of its toxic nature. In the present study response surface methodological optimization strategy was used for biosorption of Cr(VI) ions by *Azolla Filiculoidus*. Effect of various operating parameters such as initial pH of solution, initial concentration of solution, biomass dosage and temperature was studied and optimized using response surface methodology. Using central composite design, 30 experiments were carried out for the four test variables. From the statistical analysis of the experimental data the optimum condition for maximum removal of metal ions was achieved at pH-2.9, initial metal concentration-39.04, biomass dosage-0.3 g/L and temperature-33.34 ^{0}C. At these optimized conditions, the maximum percentage of chromium removal was found to be 89.24. A high R^2 value of 0.9906 indicates the fitness of the model to predict the experimental data
Keywords: Response surface methodology, central composite design (CCD), *Azolla Filiculoidus*, Cr (VI) removal.

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INTRODUCTION

Effluents discarded from various process industries like bulk chemical production, fertilizer production, pigments production, steel manufacturing sectors, etc., can cause heavy metal contamination [1]. The toxic heavy metal ions are non degradable in nature, and they enter into food chain through bioaccumulation and biomagnifications [2–4]. Among many heavy metals used in industries, chromium and its compounds are widely used in leather tanning, chromium plating, metal cleaning and processing, wood preservation, alloy preparation, rust and corrosion inhibition [5, 6]. Chromium exists in two forms viz Cr(III) ions and Cr(VI) ions, among the trivalent and hexavalent forms, hexavalent chromium has been considered to be more hazardous due to its carcinogenicity and other health effects like skin allergies, stomach and intestinal bleedings, liver and kidney damage, etc. [7]. Hexavalent chromium, because of the potential toxicity effects, has been considered as the 16th toxic contaminant [8]. Chromium is present in the industrial effluents from aluminum, ink, dye, steel, textile, paint, electroplating industries, and the tannery wastewater primarily as trivalent and hexavalent forms. The World Health Organization Standard's recommended guideline value for total chromium in drinking water as 0.05 mg L^{-1}. The permissible limit for hexavalent chromium release into inland surface water is 0.1 mg L^{-1} [9–11]. Therefore, treatment of the effluents before discharge of hexavalent chromium into aquatic environments is highly indispensable.

Various methods used for removal of heavy metal ions from aqueous solutions can be ordered as chemical precipitation, ion exchange, solvent extraction, phytoextraction, ultra filtration, reverse osmosis, electrodialysis, and adsorption[12-14]. Application of such traditional treatment techniques needs enormous cost and continuous input of chemicals which becomes impracticable and uneconomical and causes further environmental damage [15, 16]. In this context, biosorption has emerged as an alternative technique with the merits of being technically simple, eco-friendly, recyclable, using a minimal volume of sludge generation, and highly economical [17, 18]. The biosorbents contain biomolecules such as the polysaccharides, proteins, etc., with specific functional groups, which are mainly responsible for Cr(VI) biosorption [19]. Different types of biosorbents such as, Laminaria japonica [20], Undaria pinnatifida [20], Porphyra haitanensis [20], Gracilaria lemaneiformis [20], Halimeda gracilis [21], Sterculia guttata shell [22], Maize corn cob [23], Ulva lactuca [24], etc., have been investigated for the removal of Cr(VI).

In the present investigation, batch experimental studies were carried out for the removal of chromium (VI) from aqueous solution using *Azolla Filiculoidus*. The experimental data points were used to develop experimental model and optimization of process parameters. The optimization of process variables was done using three factor central composite experimental designs combined with Response Surface Methodology.

MATERIALS AND METHODS

Preparation of biosorbent

Azolla Filiculoidus a fresh water macro alga was collected from Sangam Diary, Biofertilizer and Cattle field unit, Vadlamudi, of Guntur District**,** Where it is cultivated and used as biofertilizer. To remove mud and impurities it was washed first thoroughly with tap water and later with distilled water. Then it was dried in a hot air oven at 300C for two days. Then it is powdered by ball mill and the average particle size was maintained at 100 µm. Then it was stored in air tight polythene covers for further use in biosorption experiments.

Chemicals

Analytical grades of $K_2Cr_2O_7$, HCl and NaOH were purchased from Merck, India. Chromium ions were prepared by dissolving its corresponding sulphate salt in distilled water. The pH of solutions was adjusted with 0.1 N HCl and NaOH. All the experiments were repeated four times and the average values have been reported. Also, blank experiments were conducted to ensure that no biosorption was taking place on the walls of the apparatus used.

Biosorption experiments

Batch mode adsorption studies were carried out to investigate the effect of different parameters such as initial pH of the solution, adsorbate concentration, adsorbent dosage, and temperature. The solution containing adsorbate and adsorbent was taken in 250 mL capacity Erlenmeyer flasks and agitated in an orbital shaker at 180 rpm. After one hour of contact (according to the preliminary sorption dynamics tests) equilibrium was reached and the reaction mixture was centrifuged for 5 min. The residual metal concentration in the supernatant was determined by di-phenyl carbazide method after filtering the biosorbent with whatman filter paper. The amount of metal adsorbed by *Azolla Filiculoidus* was calculated from the differences between metal quantity added to the biomass and metal content of the supernatant using the following equation:

$$q_e = \left(C_0 - C_f\right) X \frac{V}{M} \qquad \text{............(1)}$$

Where q_e is the metal uptake (mg/g); C_0 and C_f are the initial and equilibrium metal concentrations in the solution (mg/L), respectively; *V* is the solution volume (mL); and *M* is the mass of biosorbent (g).

Estimation of chromium

Amount of chromium in a given sample solution was determined spectrophotometrically at 540 nm using 1, 5-diphenyl carbazide as the complexing agent32. The sample containing Cr(VI) ions was mixed with 1 ml of 3N H_2SO_4 and 1 ml of 0.25% 1, 5-diphenyl carbazide solution and made up to known volume. The absorbance at 540 nm was measured for the purple coloured solution after 10 minutes incubation. A calibration curve was drawn in the range of 5 to 50 ppm by plotting absorbance against concentration of chromium.

Central composite design (CCD)

CCD is a statistical method based on the multivariate nonlinear model that has been widely used for the optimization of process variables of adsorption and also used to determine the regression model equations and operating conditions from the appropriate experiments [25, 26]. It is also useful in studying the interactions of the various parameters affecting the process.

The CCD was applied in this present study to determine the optimum process variables for adsorption of Cr(VI) ions using *Azolla Filiculoidus*. The CCD was used for fitting a second- order model which requires only a minimum number of experiments for modeling [27, 28]. The CCD consists of a 2^n factorial runs (coded to the usual ± notation) with 2n axial runs (±a, 0, 0, …, 0), (0, ±a, 0, 0, …, 0), …, (0, 0, …, ±a) and n_c center runs (six replicates, 0, 0, 0, …, 0). The number of factors n increases the number of runs for a complete replicate of the design which is given in Eq. 2.

$$N = 2^n + 2n + n_c \qquad \text{------------------------(2)}$$

Basically the optimization process involves three major steps: (1) performing the statistically designed experiments, (2) estimating the coefficients in a mathematical model, and (3) predicting the response and checking the adequacy of the model [29, 30]. An empirical model was developed to correlate the response to the adsorption process and is based on second order quadratic model for removal of Cr(VI) ions using *Azolla Filiculoidus* [31, 32] as given by Eq. 3 in order to analyse the effect of parameter interactions.

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

where Y is the predicted response, β_0 is the constant coefficient, β_i is the linear coefficient, β_{ij} is the interaction coefficient, β_{ii} is the quadratic coefficient, and X_i, X_j are the coded values.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Optimization of the Selected Parameters Using CCD

Independent Parameters	Range and Level				
	-2	-1	0	1	2
pH(X_1)	1	2	3	4	5
Initial Concentration(mg/L)(X_2)	10	20	60	100	120
Biomass Dosage(g/L)(X_3)	0.1	0.2	0.3	0.4	0.5
Temperature(^{0}C)(X_4)	10	20	30	40	50

Table.1. Experimental range and levels of the independent parameters for chromium biosorption onto *Azolla Filiculoidus*

The experiments with different pH values of 1– 5, different lead concentrations of 20–100 mg/L, different biosorbent dosages of 0.1–0.5 g/L and different temperatures of 10–50 ^{0}C were coupled to each other and varied simultaneously to cover the combination of parameters in the CCD. The levels and ranges of the chosen independent parameters used in the experiments for the removal of chromium were given in **Table 1.** A 2^4 – factorial CCD design, with eight axial points (α= √4) and six replications at the center points (n_o=6) leading to a total number of 30 experiments (Table 2) was employed for the optimization of the parameters. The calculated regression equation for the optimization of process variables showed that percentage removal of lead (*Y*) was function of the pH (X_1), initial concentration (X_2), biosorbent loading (X_3) and temperature (X_4). Multiple regression analysis of the experimental data resulted in the following equation for the biosorption of chromium:

$$Y = 88.79 - 0.87X_1 - 2.21X_2 + 1.05X_3 + 0.61X_4 - 0.6X_1X_2 + 2.20X_1X_3 + 0.70X_1X_4 - 1.09X_2X_3 - 0.039X_2X_4 + 2.92X_3X_4 - 7.77X_1^2 - 3.13X_2^2 - 5.80X_3^2 - 3.50X_4^2$$

The coefficients of the regression model were calculated and listed in Table 3. They contain one block term, four linear, four quadratic and six interaction terms. The significance of each coefficient was determined by p-values and listed in **Table 3.** The smaller the *p*-value, the more significant was the corresponding coefficient. This implies that the linear, quadratic and interaction effects of pH, initial concentration, biomass dosage and temperature are highly significant as is evident from their respective *p*-values in (**Table 3).** The parity plot (Figure 1) showed a satisfactory correlation between the experimental and predicted values of percentage removal of chromium indicating good agreement of model data with the experimental data. The results of the second order response surface model, fitting in the form of ANOVA were shown in **Table 4**. The Fisher variance ratio, the *F*-value (= *Sr2 /Se2*), is a statistically valid measure to test the significance and adequacy of the model. The greater the *F*-value above unity, it is more certain that the factors adequately explain the variation in the data about its mean, and the estimated factor effects are real. The ANOVA of the regression model demonstrated that the model was highly significant, as is evident from the Fisher's *F*-test (*F*model = 112.98) and a very low probability value (*P*model > *F*=0.0001). The correlation coefficient (R^2) provides a measure of the models variability in the observed response values. The closer the R^2 value to 1, the stronger the model is and it predicts the response better. In this present study, the value of the correlation coefficient (R^2 = 0.9906) indicated that 99.06 % of the variability in the response could be explained by the model. In addition, the value of the adjusted correlation coefficient (Adj R^2 = 0.9818) was also very high to advocate for a high significance of the model. The response surface plots of percentage biosorption of chromium versus the interactive effect of pH, initial lead concentration, biosorbent dosage and temperature were shown **in the Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, and Figure 7.** Each response plot represents a number of combinations of two test parameters with the other parameter maintained at zero levels. The maximum percentage biosorption of level is indicated by the surface confined in the smallest curve (circular or elliptical) of the response plot. The optimal set of conditions for maximum percentage biosorption of lead is pH = 4.72, initial concentration of lead in aqueous solution = 58.523 mg/L, biosorbent dosage = 0.271 g/L and temperature = 39.230C. The extent of biosorption of lead at these optimum conditions was 83.775%. The optimum values of variables for lead biosorption from regression equation were shown **in table 5.**

Run No.	Coded Values of Process Variables				% Removal	
	pH (X_1)	Initial Concentration(mg/L) (X_2)	Biomass dosage(g/L) (X_3)	Temperature (^{0}C) (X_4)	Observed Values	Predicted Values
1	-1	-1	-1	-1	74.89	74.09
2	0	0	0	0	88.79	67.76
3	1	1	1	1	70.29	73.14
4	0	0	0	-2	74.51	64.39
5	0	2	0	0	73.46	68.13
6	1	-1	1	-1	70.91	70.60
7	2	0	0	0	57.52	62.81
8	-1	1	1	1	67.78	62.86
9	0	0	0	0	88.79	68.16
10	-1	1	-1	-1	72.93	64.63
11	1	-1	-1	1	65.46	67.05
12	0	0	2	0	69.14	61.10
13	1	-1	1	1	77.28	73.86
14	0	-2	0	0	81.34	79.14
15	0	0	0	0	88.79	68.39
16	-1	1	-1	1	66.59	71.25
17	1	-1	-1	-1	66.31	59.45
18	0	0	0	2	77.29	55.97
19	0	0	-2	0	64.25	80.70
20	0	0	0	0	88.79	71.86
21	0	0	0	0	88.79	63.48
22	-1	-1	1	1	74.56	67.67
23	0	0	0	0	88.79	73.55
24	1	1	1	-1	62.37	76.01
25	1	1	-1	1	60.28	88.79
26	-2	0	0	0	60.14	88.79
27	-1	-1	-1	1	66.59	88.79
28	-1	-1	1	-1	66.89	88.79
29	1	1	-1	-1	63.54	88.79
30	-1	1	1	-1	61.83	88.79

Table 2: CCD matrix showing coded values of process variables along with the observed and predicted values for percentage biosorption of chromium with *Azolla Filiculoidus*

Term	Coefficient	Value	Standard error of coefficient	F-value	p-value
Constant	β_0	88.79	0.545639	112.9777	< 0.0001^a
X_1	β_1	-0.869	0.27282	10.14975	0.0061^a
X_2	β_2	-2.21	0.27282	65.6196	< 0.0001^a
X_3	β_3	1.045	0.27282	14.69515	0.0016^a
X_4	β_4	0.613	0.27282	5.05408	0.0400^a
X_1X_2	β_{12}	-0.605	0.334134	3.27845	0.0903
X_1X_3	β_{13}	2.2	0.334134	43.3514	< 0.0001^a
X_1X_4	β_{14}	0.7	0.334134	4.388881	0.0536
X_2X_3	β_{23}	-1.091	0.334134	10.66612	0.0052^a
X_2X_4	β_{24}	-0.038	0.334134	0.013449	0.9092
X_3X_4	β_{34}	2.916	0.334134	76.17409	< 0.0001^a
X_1^2	β_{11}	-7.769	0.255199	926.8081	< 0.0001^a
X_2^2	β_{22}	-3.126	0.255199	150.1081	< 0.0001^a
X_3^2	β_{33}	-5.802	0.255199	517.0513	< 0.0001^a
X_4^2	β_{44}	-3.501	0.255199	188.2741	< 0.0001^a

A= pH, B= Initial Concentration, C= Biosorbent dosage, D= Temperature. ^{a}Significant ($p \leq 0.05$)

Table.3. Coefficients and significance probability of the model for biosorption of chromium onto *Azolla Filiculoidus*

Source of variation	Sum of squares (SS)	Degrees of freedom (D.F)	Mean squares (MS)	F-value	P-Value Probe>F
Model	2825.42	14	201.82	112.98	0.0001
Error	0.000	5	0.000		
Total	2852.22	29			

R^2= 0.9906; Adjusted R^2= 0.9819

Table.4. ANOVA for the entire quadratic model for biosorption of chromium onto *Azolla Filiculoidus*

Parameter	Optimum value for chromium
pH	2.9
Initial Concentration(mg/L)	39.04
Biomass Dosage(g/L)	0.38
Temperature(^{0}C)	33.34

Table.5. Optimum values of variables obtained from regression equations for the removal of chromium onto *Azolla Filiculoidus*

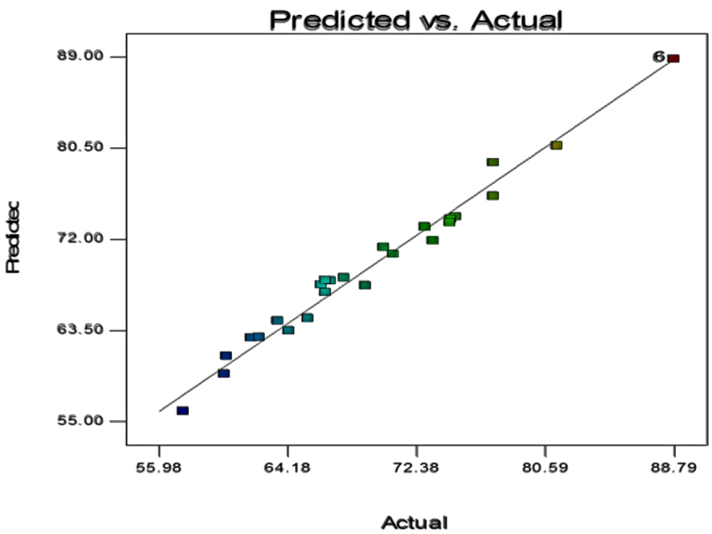

Figure.1. Parity plot showing the distribution of actual vs. predicted values of percentage biosorption of Chromium onto *Azolla Filiculoidus*

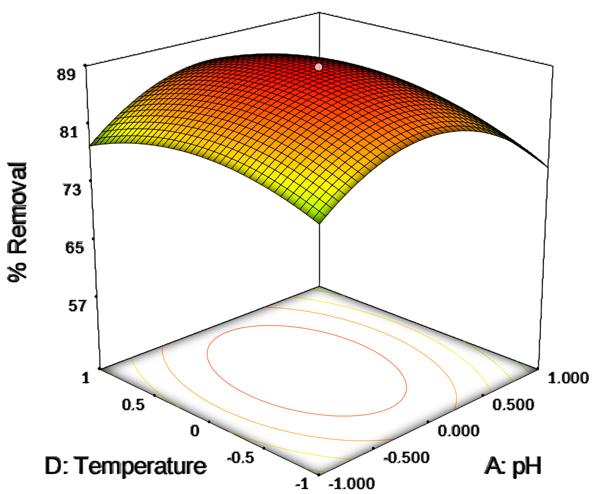

Figure.2. Response surface plot of the effects of pH and temperature on percentage biosorption of Chromium onto *Azolla Filiculoidus*

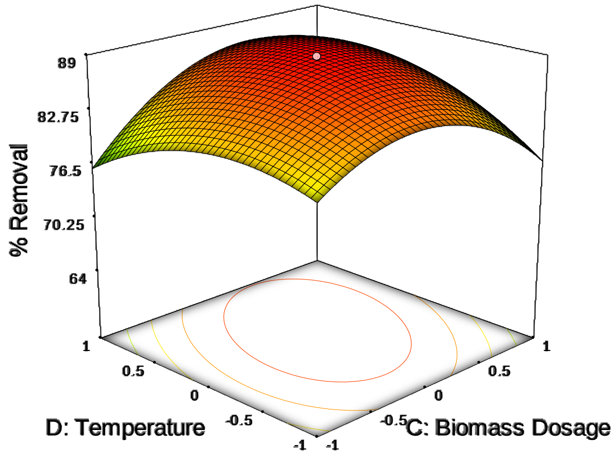

Figure.3. Response surface plot of the effects of biosorbent dosage and temperature on percentage biosorption of Chromium onto *Azolla Filiculoidus*

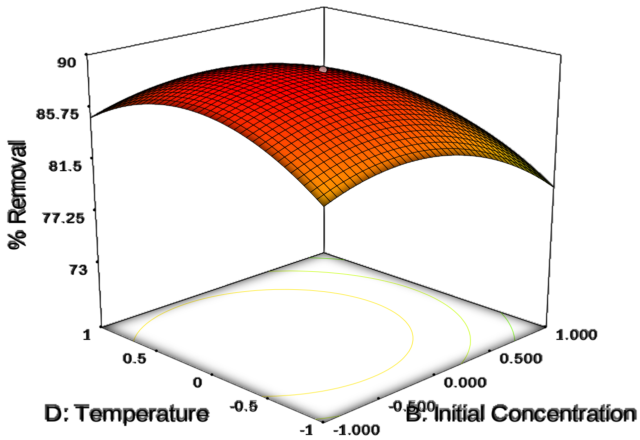

Figure.4. Response surface plot of the effects of inicial metal concentration and temperature on percentage biosorption of Chromium onto *Azolla Filiculoidus*

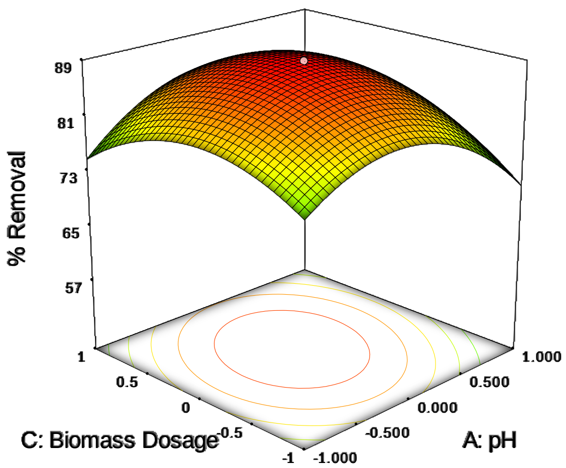

Figure.5. Response surface plot of the effects of biosorbent dosage and pH on percentage biosorption of Chromium onto *Azolla Filiculoidus*

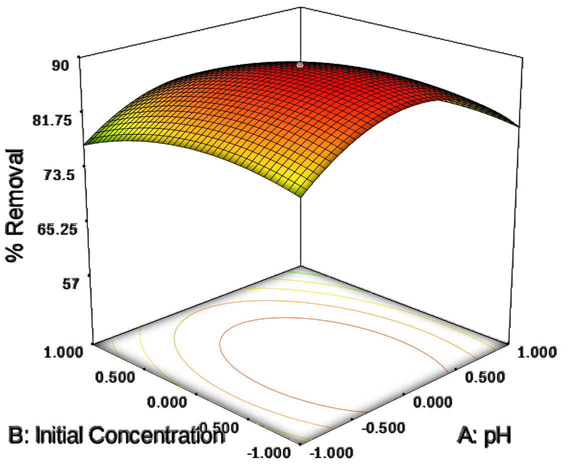

Figure.6. Response surface plot of the effects of initial metal concentration and pH on percentage biosorption of Chromium onto *Azolla Filiculoidus*

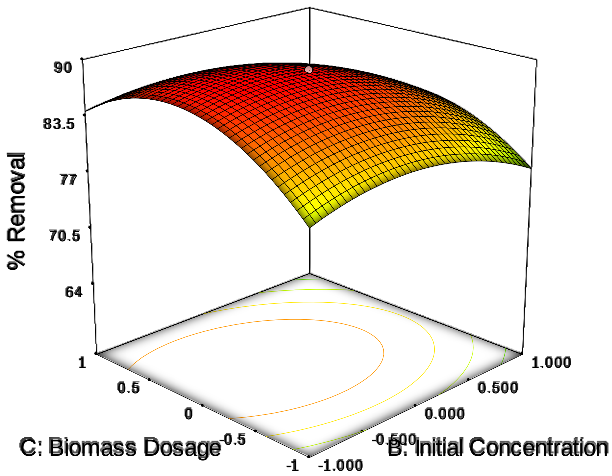

Figure.7. Response surface plot of the effects of initial metal concentration and biosorbent dosage on percentage biosorption of Chromium onto *Azolla Filiculoidus*

CONCLUSIONS

A detailed batch experimental study was carried out for the removal of Cr (VI) from waste water by using *Azolla Filiculoidus*. This work has demonstrated the use of a full factorial central composite design by determining the optimum process conditions leading to the maximum percentage removal of Cr(VI) from aqueous solutions. Response surface methodology using CCD proved very effective and time saving model for studying the influence of process parameters on response factor by significantly reducing the number of experiments and hence facilitating the optimum conditions. Using this experimental design and multiple regressions, the parameters namely, temperature, pH, biosorbent loading, initial lead ion concentration were studied effectively and optimized The Experimental values and the predicted values are in perfect match with R^2 value of 0.996. This methodology could therefore be successfully employed to study the importance of individual, cumulative, and interactive effects of the test variables in biosorption.

REFERENCES

[1] Zouboulis AI, Loukidou MX, Matis KA. Process. Biochem. 2004; 39: 909–916

[2] Ahmed MS, Aslam Y, Khan WA. J. Anim. Plant Sci. 2011; 21: 176–181

[3] Mortazavi MS, Sharifian S. Int. J. Environ. Res. 2011; 5: 757–762

[4] Ahmed MS, Bibi S. J. Anim. Plant Sci. 2010; 20: 201–207
[5] Jacques RA, Lima EC, Dias SLP, Mazzocato AC, Pavan FA. Sep Purif Technol 2007; 57: 193–198
[6] Mohanty K, Jha M, Meikap BC, Biswas MN. Chem Eng J 2006; 117: 71–77
[7] Nameni M, Moghadam MRA, Arami M. Int J Environ Sci Technol 2008; 5:161–168
[8] Kowalski ZJ. J. Hazard. Mater. 1994; 37: 137–144
[9] WHO, Guidelines for Drinking-Water Quality (World Health Organization, Geneva, 1993)
[10] IS 10500:1991 (1991) Drinking Water Specifications. Bureau of Indian Standards, New Delhi
[11] Owlad M, Aroua MK, Daud WAW, Baroutian S. Water Air Soil Pollut. 2009; 200: 59–77
[12] Patterson JW, Industrial Wastewater Treatment Technology, 2nd ed., Butterworth– Heinemann, London, 1985.
[13] Bhattacharya KA, Mandal SN, Das SK. *Chem. Eng. J.* 2006; 123: 43–51.
[14] John Babu D, Sumalatha B, Venkateswrulu TC, Mariya Das K Vidya PK. J Pure and Appl. Microbiol. 2014; 8(4) : 3107 – 3116
[15] Prakasham RS, Sheno Merrie J, Sheela R, Saswathi N, Ramakrishna S.V. *Environ. Pollut.* 1999; 104: 421–427
[16] John Babu D, Prasanna Kumar Y, King P. International Journal of ChemTech Research, 2014; 6(14): 5535-5545,
[17] Gupta VK, Rastogi A. J. Hazard. Mater. 2008; 152: 407–414
[18] Rangabhashiyam S, Suganya E, Selvaraju N, Lity AV. World J. Microbiol. Biotechnol. 2014; 30: 1669–1689
[19] Marina F, Geoffrey MG, Bioresour. Technol. 2014; 160: 3–14
[20] Rangabhashiyam S, Selvaraju N. J. Taiwan Inst. Chem. Eng. 2015; 47: 59–70
[21] Cheng-guang J, Ya-ping Z, He W, Guang-nan O, Qi-ming L, Jin-mei L, J. Cent, South Univ. 2014; 21: 2801–2809
[22] Jayakumar R, Rajasimman M, Karthikeyan C, J. Environ. Chem. Eng. 2014; 2: 1261–1274
[23] Rangabhashiyam S, Selvaraju N. J. Mol. Liq. 2015; 207, 39–49
[24] Sikaily AE, Ei NA, Khaled A, Abdelwehab O. J. Hazard. Mater. 2007; 148: 216–228
[25] Kalavathy HM, Regupathi I, Pillai MG, Miranda LRColloids Surf B. 2009; 70:35–45
[26] Arulkumar M, Sathishkumar P, Palvannan T. J Hazard Mater 2011; 186: 827–834
[27] Ahmad AA, Hameed BH, Ahmad AL. J Hazard Mater. 2009; 170:612–619
[28] Tanyildizi MS. Chem Eng J. 2011; 168:1234–1240
[29] Sahu JN, Acharya J, Meikap BC. J Hazard Mater 2009; 172:818–825
[30] Kumar R, Singh R, Kumar N, Bishnoi K, Bishnoi NR. Chem Eng J . 2009; 146:401–407
[31] Bashir MJK, Aziz HA, Yusoff MS, Adlan MN. Desalination 2010; 254:154–161
[32] Dutta S, Bhattacharyya A, Ganguly A, Gupta S, Basu S. Desalination 2011; 275:26–36