*Int. J. Chem. Sci.: 12(1), 2014, 169-180*
*ISSN 0972-768X*
*www.sadgurupublications.com*

# STATISTICAL OPTIMIZATION ON CHROMIUM (VI) REDUCTION BY MARINE BACTERIA, *Planococcus* sp. VITP21 USING CANE SUGAR AS CARBON SOURCE

**SANGEETHA SUBRAMANIAN*, MRUDULA PULIMI and GURUNATHAN JAYARAMAN**

School of Biosciences and Technology, VIT University, VELLORE – 632014 (T.N.) INDIA

## ABSTRACT

Cr (VI) reduction capacity of marine bacteria, *Planococcus* sp.VITP21 from Kumta coast of Karnataka, India was optimized using statistical design technique under saline condition. Plackett Burman design was used for preliminary screening of important physical and chemical parameters that influenced Cr (VI) reduction. The four parameter pH, Cr (VI) concentration, cane sugar (a carbon source) and NaCl concentration were chosen for optimization using central composite design. The optimized combination of parameters was found to be 2.83 g/L cane sugar, 6.8 pH, 142.8 mg/L Cr (VI) concentration and 8% w/v NaCl with 83% of reduction efficiency in 12 hours of incubation. *Planococcus* sp.VITP21 was shown to have enhanced reduction potential under saline condition.

**Key words**: Cr (VI) reduction, *Planococcus* sp. VITP21, Saline condition, Plackett Burman, Response surface methodology and central composite design.

## INTRODUCTION

Industrial waste water (leather and textile industry) generated due to anthropogenic activities contains toxic and non essential heavy metals like chromium[1], in higher concentration along with salt (NaCl). Although chromium exists in different oxidation states, hexavalent and trivalent chromium are the stable ones. Compared to trivalent chromium, hexavalent chromium is highly toxic and carcinogenic and hence recommended by EPA for its permissible level in drinking water as 0.05 mg/L[2]. Conventional water treatment methods like reduction and precipitation by chemical agents generates larger quantities of sludge[3], moreover adsorption/biosorption disposes the chromium in the form of hexavalent form only. Hence the other alternative method is bacterial mediated chromium reduction, which

*Author for correspondence; E-mail: sangeethasubramanian@vit.ac.in

converts toxic Cr (VI) to non toxic Cr (III). Many reports have investigated the role of bacteria in chromium reduction under non saline condition[4-6]. Under saline conditions, only a few bacteria were reported on Cr (VI) reduction but with the traditional optimization approach (one variable at a time)[2,7]. Optimization and study on effect of different physico-chemical parameters on Cr (VI) reduction becomes important so as to employ a microorganism for chromium remediation in the effluent under different environmental condition[8]. The traditional one variable at a time optimization method does not depicts the effect of different variable on Cr (VI) reduction, factorial experimental design like Plackett Burman and Response surface methodology would be the alternate solution[9,10]. Henceforth, the present study used Plackett Burman to identify the important physico-chemical parameters that influences Cr (VI) reduction and RSM was used further to optimize and to study the effect of important variables on Cr (VI) reduction by *Planococcus* sp. VITP21 using cane sugar as carbon source under saline condition.

## EXPERIMENTAL

### Microorganism and growth conditions

*Planococcus* sp. VITP21 (GenBank accession No: HQ829427), chromium resistant bacteria isolated from Kumta coast, Karnataka, India was used for the present investigation[11]. *Planococcus* sp. VITP21 was grown in Luria-Bertani medium with 4% (w/v) NaCl (Temperature 35ºC, pH 7.0 and agitation rate- 140 rpm) under aerobic condition, unless otherwise stated. The pH of the media was adjusted to 7.0 with either 1 M NaOH or HCl. The Cr (VI) was added as potassium dichromate and all experiments were performed in duplicates under identical conditions.

### Design of experiments for Cr (VI) reduction by Planococcus sp.VITP21

### Plackett Burman (PDB) design

Plackett Burman design (PDB) was used to assess important physico-chemical variables that influences Cr (VI) reduction. The variables considered were initial Cr (VI) concentration, pH, temperature, Incubation time, Inoculum concentration, NaCl concentration and agitation rate (rpm). The experiments were performed in LB medium. The design screened for 11 variables with 12 experimental runs to evaluate 7 variables and 4 variables were assigned as dummy variables. The analysis was carried out in two levels, low level (-1) and high level (+1) and the list of variables used for screening and levels of each variable are given in Table 1. Table 2 details the experimental design. All experiments were performed in duplicates and average values were considered as response.

**Table 1: Variables and their concentration in Plackett-Burman design**

| Variable | Low level (-1) | High value (+1) |
|---|---|---|
| pH | 6 | 8 |
| Temperature (Centigrade) | 35 | 45 |
| NaCl (% w/v) | 1 | 6 |
| Cr(VI) mg/L | 100 | 200 |
| Inoculum (% v/v) | 1 | 3 |
| Agitation rate (rpm) | 140 | 180 |
| Time (h) | 24 | 48 |

**Table 2: Plackett Burman design**

| Run | Variable | | | | | | | | | | | (%) Cr (VI) Reduction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | X-1 | X-2 | X-3 | X-4 | |
| 1 | 1 | 1 | -1 | 1 | 1 | 1 | -1 | -1 | -1 | 1 | -1 | 41.2 |
| 2 | -1 | 1 | 1 | -1 | 1 | 1 | 1 | -1 | -1 | -1 | -1 | 0 |
| 3 | 1 | 1 | 1 | -1 | -1 | -1 | 1 | -1 | 1 | 1 | -1 | 89.5 |
| 4 | -1 | 1 | -1 | 1 | 1 | -1 | 1 | 1 | 1 | -1 | -1 | 0 |
| 5 | 1 | 1 | -1 | -1 | -1 | 1 | -1 | 1 | 1 | -1 | 1 | 63.89 |
| 6 | 1 | -1 | -1 | -1 | 1 | -1 | 1 | 1 | -1 | 1 | 1 | 77.22 |
| 7 | -1 | -1 | 1 | -1 | 1 | 1 | -1 | 1 | 1 | 1 | -1 | 0 |
| 8 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | 4 |
| 9 | -1 | 1 | 1 | 1 | -1 | -1 | -1 | 1 | -1 | 1 | 1 | 0 |
| 10 | -1 | -1 | -1 | 1 | -1 | 1 | 1 | -1 | 1 | 1 | 1 | 0 |
| 11 | 1 | -1 | 1 | 1 | -1 | 1 | 1 | 1 | -1 | -1 | -1 | 66.8 |
| 12 | 1 | -1 | 1 | 1 | 1 | -1 | -1 | -1 | 1 | -1 | 1 | 39.55 |

Variable : A, pH; B, Temperature; C, NaCl; D, Cr(VI); E, Inoculum; F, RPM; G; Time

## Response surface methodology

Four variables pH, Cr (VI) concentration, cane sugar and NaCl were studied by central composite design. Imperative independent variable (pH and Cr (VI) concentration) were determined from Plackett-Burman experiments for Cr (VI) reduction. Based on screening for carbon source for Cr (VI) reduction, cost effective cane sugar (a cottage jaggery) was selected as another variable (Data not shown). The jagerry, a concentrated cane sugar juice produced by cottage industries in India was used. Finally in order to study the interaction of salt (NaCl) during Cr (VI) reduction due to the fact that organism was from marine source, NaCl was selected as one among the four variables. Optimization of these variables was performed by central composite design (CCD) and response surface methodology. The details of experimental variables, range and their levels were presented in Table 3. A $2^4$ full-factorial experimental with 5 replicates at the center point and 21 total experiments were engaged in the experiments. The center position discloses the degree of inaccuracy in the experimentation. Table 4 gives the detail of experimental chart for CCD experiment.

**Table 3: Levels of variable used in central composite design for maximum Cr (VI) reduction**

| Variable | Low actual | High actual | Low coded | High coded |
|---|---|---|---|---|
| A : Cane sugar (g/L) | 1 | 3 | -1 | 1 |
| B : pH | 5 | 8 | -1 | 1 |
| C : Cr(VI) (mg/L) | 100 | 300 | -1 | 1 |
| D : NaCl (% wt.) | 6 | 10 | -1 | 1 |

**Table 4: Experimental results of central composite design on Cr (VI) reduction by *Planococus* sp. VITP21**

| A : Cane sugar | B : pH | C : Cr (VI) | D : NaCl | Actual Cr (VI) reduction (%) | Predicted Cr (VI) reduction (%) |
|---|---|---|---|---|---|
| 1 | 5 | 100 | 6 | 68.09 | 70.70 |
| 3 | 5 | 100 | 10 | 66.54 | 68.89 |
| 2 | 6.5 | 368.18 | 8 | 45.82 | 45.97 |
| 3 | 5 | 300 | 10 | 57.47 | 53.05 |
| 3 | 8 | 100 | 6 | 75.94 | 78.36 |
| 2 | 9.02 | 200 | 8 | 67.63 | 65.16 |
| 1 | 8 | 300 | 10 | 62.41 | 58.12 |

Cont…

| A : Cane sugar | B : pH | C : Cr (VI) | D : NaCl | Actual Cr (VI) reduction (%) | Predicted Cr (VI) reduction (%) |
|---|---|---|---|---|---|
| 1 | 8 | 100 | 10 | 73.53 | 75.88 |
| 3 | 8 | 300 | 6 | 65.92 | 61.68 |
| 2 | 6.5 | 200 | 4.64 | 81.68 | 79.34 |
| 2 | 6.5 | 200 | 8 | 80.38 | 79.50 |
| 2 | 6.5 | 200 | 8 | 80.77 | 79.50 |
| 2 | 6.5 | 200 | 8 | 80.34 | 79.50 |
| 2 | 6.5 | 31.82 | 8 | 80.20 | 73.42 |
| 2 | 6.5 | 200 | 11.36 | 78.66 | 76.04 |
| 0.32 | 6.5 | 200 | 8 | 75.13 | 72.75 |
| 2 | 6.5 | 200 | 8 | 79.87 | 79.50 |
| 3.68 | 6.5 | 200 | 8 | 80.17 | 77.66 |
| 2 | 3.98 | 200 | 8 | 67.80 | 65.38 |
| 2 | 6.5 | 200 | 8 | 80.34 | 79.50 |
| 1 | 5 | 300 | 6 | 59.85 | 55.69 |

The performance of the organism with respect to Cr (VI) reduction was explained by the following Quadratic equation:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\, \beta_{ii} x_i^2 + \Sigma\, \beta_{ij}\, x_i\, x_j \qquad \text{....(1)}$$

where, Y = Predicted response, β = Coefficients and x = variables

The variables were based on the following equation:

$$x_i = X_i - X / \Delta X \qquad \text{...(2)}$$

where, $x_i$ = Coded value, $X_i$ = Actual value, X = Average value of the high and low concentrations, and ΔX = Tread change.

All the experiments were conducted with 4 g/L of yeast extract as a nitrogen source and temperature maintained at 35ºC with agitation rate of 140 rpm. The experiments were designed and analyzed by statistical software package Design-Expert (Version 6.0.10; State-Ease, Minneapolis, MN, USA).

## Analytical method

In all experiments, concentration of hexavalent chromium was determined by diphenylcarbazide method[12]. Accordingly, the hexavalent chromium was determined spectrophotometrically by reaction with diphenylcarbazide in acid solution (6 M $H_2SO_4$). Cell free supernatant was made upto 1 mL using distilled water followed by addition of 330 µL of 6 M $H_2SO_4$ and 400 µL of diphenylcarbazide (0.25% w/v in acetone). The solution was diluted to 10 mL using distilled water. The absorbance of the samples was read at 540 nm to determine the concentration of hexavalent chromium.

# RESULTS AND DISCUSSION

## Plackett Burman design

The Plackett Burman design experiment is the influential tool to screen significant parameters that have influence on Cr (VI) reduction[13]. The physico-chemical variables like pH, temperature, NaCl, Cr (VI), Inoculum, agitation rate and time of incubation were tested for their significance in LB media for maximum Cr (VI) reduction. The analysis of experimental results indicated wide variation in Cr (VI) reduction Table 2.

The Table 5 depicts the results of an analysis of variance (ANOVA) for the experiment. The higher F-value (33.8) and corresponding lesser P-value (0.0021) indicated that the model was significant[13]. Values of "Prob > F" less than 0.05 indicated that the model terms were significant. The coefficient of determination ($R^2$) 0.98 was found to be high for the model studied. The effect of variables was discussed based on model obtained from regression analysis and presented in the pareto chart in the Fig. 1.

$$\text{Cr (VI) reduction (\%)} = + 31.85 + 31.18 * A + 0.59 * B + 0.79 * C - 7.25 * D - 5.52 * E - 3.20\ F + 7.07 * G$$

Variable A, G, B and C indicates positive effect; D, E and F indicates negative effect in the present investigation for Cr (VI) reduction, pH, Cr (VI) and time were identified to be a significant model terms. The positive and negative value of coefficient indicated the increasing and decreasing requirement of corresponding concentration/range for maximum Cr (VI) reduction[14]. The pH and time showed positive effect whereas Cr (VI) showed negative effect on Cr (VI) reduction. The effect of NaCl was found to be insignificant for a marine *Planococcus* sp.VITP21 to reduce Cr (VI), however NaCl shows a positive effect. Therefore for enhancing Cr (VI) reduction highly significant variables were identified to be pH and Cr (VI), which were selected for further optimization.

**Table 5: Anova results for Plackett Burman Cr (VI) reduction design Experiment**

| Variable | Mean square | F- Value | Prob > F |
|---|---|---|---|
| Model | 1914 | 33.8 | 0.0021 |
| A: pH | 11666 | 206.22 | 0.0001 |
| B : Temperature | 4.11 | 0.073 | 0.8009 |
| C : NaCl | 7.58 | 0.13 | 0.7328 |
| D : Cr (VI) | 6.32E + 02 | 11.16 | 0.0288 |
| E : Inoculum | 365.42 | 6.46 | 0.0639 |
| F : RPM | 122.75 | 2.17 | 0.2147 |
| G : Time | 600.38 | 10.61 | 0.031 |
| $R^2$=0.9834 | | | |

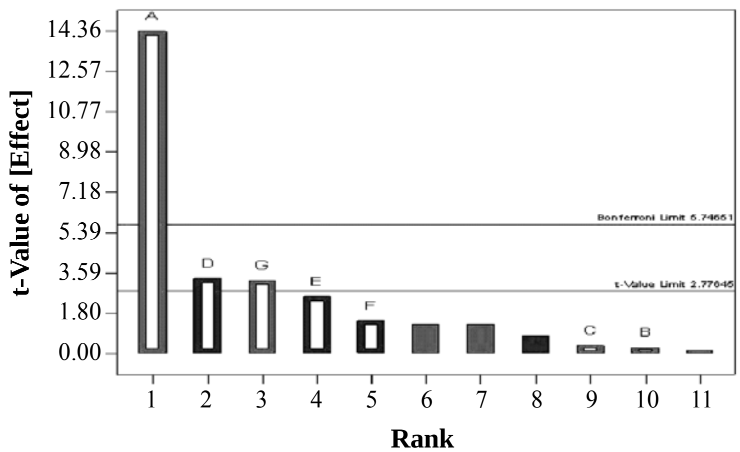



**Fig. 1: Pareto chart for Plackett Burman Cr (VI) reduction experiment**

## Central Composite Design (CCD)

The interaction between four variables: cane sugar, pH, Cr (VI) and NaCl on Cr (VI) reduction capacity was resoluted by response surface methodology using central composite design (CCD). The Table 4 shows the results of actual and predcited value for CCD experiments which ranges with Cr (VI) reduction capacity of 46% to 82%. The statistical significance of the results of central composite design was evaluated by ANOVA analysis (Table 6). The F-value of 6.79 and P value of 0.01 depicted that the model was significant. The model terms C, $B^2$ and $C^2$ contributed for the significance of the model. The coefficient of determination ($R^2$) was found to be 0.94 confirming that model to be significant[15]. For predicting the best possible point within the experimental limit the following quadratic equation was used:

$$\% \text{ Cr (VI) reduction} = -49.09 + 19.17.50*A - 25.88*B - 0.23.05*C - 2.86*D - 1.52*A2 - 2.24*B2 - 0.00067*C2 - 0.1562*D2 + 0.223339*A*B + 0.000323*A*C - 1.64021*A*D - 0.00318*B*C + 0.41716*B*D - 0.0012*C*D$$

Where A, B, C and D are cane sugar, pH, Cr (VI) and NaCl, repectively. The relationship between Cr (VI) reduction capacity and the variables was obtained by the equation and the Fig. 2 represents predicted versus actual values. The pattern of graph showed that the predicted and the experimental values were congregated around the central diagonal line confirming the corelation between the same.

**Table 6: ANOVA analysis for Cr (VI) reduction capacity (%) for central composite design**

| Term | DF[a] | MS[b] | F-Value | Prob > F | |
|---|---|---|---|---|---|
| Model | 14 | 127.8004 | 6.786399 | 0.0134 | Significant |
| A | 1 | 12.71645 | 0.675263 | 0.4426 | |
| B | 1 | 0.014863 | 0.000789 | 0.9785 | |
| C | 1 | 678.6566 | 36.03772 | 0.0010 | |
| D | 1 | 4.551873 | 0.241712 | 0.6404 | |
| $A^2$ | 1 | 34.35855 | 1.824492 | 0.2255 | |
| $B^2$ | 1 | 377.9434 | 20.06938 | 0.0042 | |
| $C^2$ | 1 | 669.3192 | 35.54189 | 0.0010 | |
| $D^2$ | 1 | 5.833587 | 0.309773 | 0.5979 | |
| AB | 1 | 0.371901 | 0.019749 | 0.8928 | |
| AC | 1 | 0.008361 | 0.000444 | 0.9839 | |
| AD | 1 | 35.65945 | 1.893572 | 0.2180 | |
| BC | 1 | 1.825807 | 0.096953 | 0.7661 | |
| BD | 1 | 5.189928 | 0.275593 | 0.6184 | |
| CD | 1 | 0.463341 | 0.024604 | 0.8805 | |
| Residual | 6 | 18.83184 | | | |
| Pure Error | 4 | 0.103114 | | | |
| Cor Total | 20 | | | | |

[a]Degrees of freedom; [b]Mean square; $R^2$-0.9406

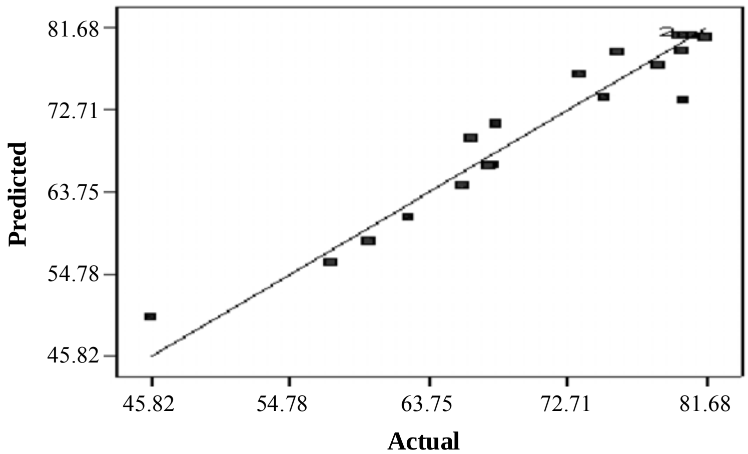
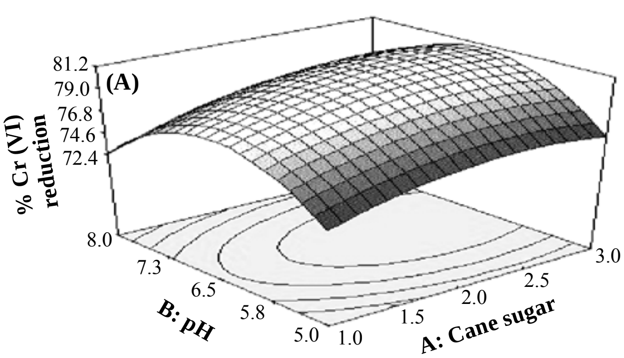



**Fig. 2: Predicted versus actual values plot**

## Interaction effects of variables by response surface plots

The interaction effect between different variables (cane sugar, pH, Cr (VI) and NaCl) were studied for maximum Cr (VI) reduction by response surface plots. Fig. 3 shows the response surface of different variable and their interaction. The figure A shows the interaction of cane sugar and pH with elliptical contours which implies that the interaction was significant. As the pH increased from pH 5, the reduction capacity increased and then decreased showing the optimum around pH 6.5. With the figure B, the interaction between Cr (VI) concentration and pH was studied, which showed that the increase in Cr (VI) concentration increased reduction capacity upto 200 mg/L. The effect of pH with Cr (VI) also showed optimum reduction capacity around 6.5. The effect pH with NaCl was presented in Figure C. The increase in NaCl did not show any significant change in reduction capacity, however showing slight decrease in reduction capacity at higher salt concentration. The pH showed same effect as with other variable with optimum around neutral pH.




Cont…

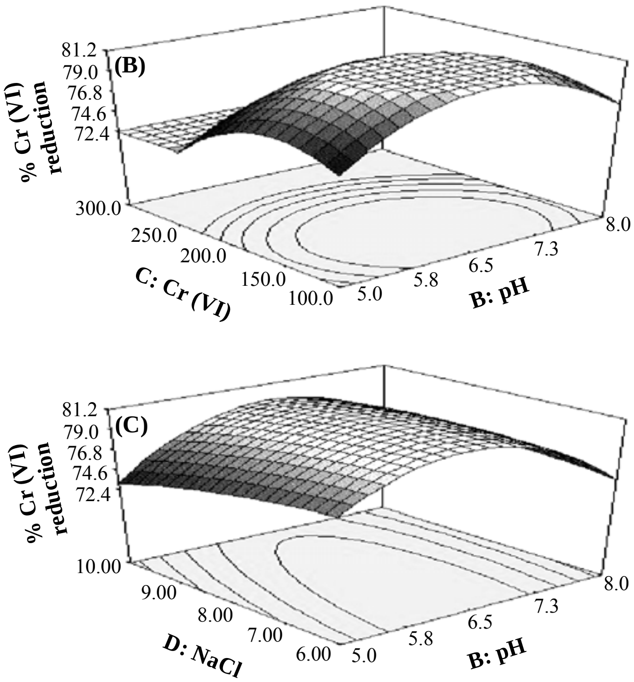



**Fig. 3: Three dimensional plot of Cr (VI) reduction by *Planococcus* sp. VITP21 (A) Cane sugar with pH (B) pH with Cr (VI) (C) pH with NaCl**

## Optimization on Cr (VI) by Planococcus sp. VITP21

In order to increase the reduction efficiency, the optimal conditions were identified by the Design-Expert software. 2.83 g/L cane sugar, 6.8 pH, 142.8 mg/L Cr (VI) concentration and 8% w/v NaCl with 83% of reduction efficiency in 12 hrs of incubation were the optimal solution obtained by the program. The optimal solution was based on the goal to obtain maximum Cr (VI) reduction in 12 h of incubation using lowest concentration of cost effective carbon source. The desirability factor of 1 was obtained for the experiment which indicated that the process had maximum efficiency under the operating conditions. Sathiyanarayanan et al.[16] reported on a statistical approach for the optimization of polyhydroxybutyrate production by marine *Bacillus subtilis* MSBN17 using jaggery as carbon source. Cr (VI) reduction under non saline condition was reported by Pulimi et al.[14] using molasses as carbon source with 99.9% reduction of 54 mg/L of Cr (VI) in 12 hrs of incubation. Mishra et al.[2] reported 99% Cr (VI) reduction of 100 mg/L in Luria Bertani media with additional carbon source as glucose within 76 hrs of incubation under saline condition (6% (w/v) NaCl). Marine *Planococcus* sp. VITP21 reduced maximum of 83% of 142.8 mg/L in 12 hr of incubation using cane sugar as carbon source under saline condition.

In the present investigation, Plackett-Burman design and response surface methodology were successfully applied for the fast screening and optimization of operational parameters for chromium reduction. Thus the desired goal of increase in reduction rate with economically feasible carbon source (cane sugar) was achieved for marine bacteria, *Planococcu*s sp. VITP21 under high saline condition. This is the first statistical optimization study reported to our knowledge under the saline condition for Cr (VI) reduction by a marine bacteria.

## ACKNOWLEDGEMENT

The authors wish to thank the management of VIT University for providing the necessary support for carrying this study.

## REFERENCES

1. B. Dhal, H. N. Thatoi, N. N. Das and B. D. Pandey, J. Hazard. Mater., **250-251**, 272-291 (2013).
2. R. R. Mishra, B. Dhal, S. K. Dutta, T. K. Dangar, N. N. Das and H. N. Thatoi, J. Hazard. Mater., **227-228**, 219-226 (2012).
3. C. E. Barrera-Díaza, V. Lugo-Lugo, B. Bilyeu, J. Hazard. Mater., **223-224**, 1-12 (2012).
4. S. Dey and A. K. Paul. J. Hazard. Mater., **213-214**, 200-206 (2012).
5. L. Xu, M. Luo, W. Li, X. Wei, K. Xie, L. Liu, C. Jiang and H. Liu, J. Hazard. Mater., **185**, 1169-1176 (2011).
6. S. Ge, M. Zhou, X. Dong, Y. Lu and S. Ge. Appl. Microbiol. Biotechnol., **97**, 2131-2137 (2013).
7. M. A. Amoozegar, A. Ghasemi, M. R. Razavi and S. Naddaf, Proc. Biochem., **42**, 1475-1479 (2007).
8. S. Dey and A. K. Paul, J. Hazard. Mater., **213-214**, 200-206 (2012).
9. S. W. Cheng, Y. F. Wang and M. L. Wang, Chem. Biochem. Eng. Q., **26**, 225-231 (2012).
10. K. Ravikumar, B. Deebika and K. Balu, J. Hazard. Mater., **122**,75-83 (2005).
11. S. Subramanian, S. Sam and G. Jayaraman, Afr. J. Microbiol. Res., **6**, 7339-7349 (2012).

12. U. Thacker, R. Parikh, Y. Shouche and D. Madamwar, Pros. Biochem., **41**, 1332-1337 (2006).

13. D. Gunasundari and K. Muthukumar, Environ. Sci. Pollut. Res. Int., **20**, 6563-6573 (2013).

14. Pulimi, Mrudula, S. Jamwal, J. Samuel, N. Chandrasekaran and A. Mukherjee, J. Microbiol. Biotechnol., **22**, 1767-1775 (2012).

15. M. Pazouki, M. Keyanpour-Rad, S. Shafie and S. H. Shahhoseini, Bioresour. Technol., **98**, 2116-2122 (2007).

16. G. Sathiyanarayanan, G. Saibaba, G. Seghal Kiran and Joseph Selvin, Int. J. Biol. Macromol., **59**, 170-177 (2013).

*Accepted : 13.11.2013*