INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

OPTIMIZATION OF PHENOL DEGRADATION FROM *Pseudomonas aeruginosa* (NCIM 2074) USING RESPONSE SURFACE METHODOLOGY

Chandana Lakshmi MVV[1*], Sridevi V[1], Narasimha Rao M[2] and Swamy AVN[3]

[1]Centre for Biotechnology, Department of Chemical Engineering, Andhra University, Visakhapatnam, Andhra Pradesh, India.

[2]Al-Ameer College of Engineering and Information Technology, College of Engineering, Gudilova, Anandapuram, Visakhapatnam, Andhra Pradesh, India.

[3]Department of Biotechnology, J.N.T.U, Pulivendula, Anantapur Dist., Andhra Pradesh, India.

***Corresponding Author: mahantilakshmi@yahoo.com**

ABSTRACT

In the present investigation, *Pseudomonas aeruginosa* (NCIM 2074) which can utilize phenol as a sole source of carbon and energy was selected for the degradation of phenol. Experiments were made as a function of carbon source (glucose), inorganic nitrogen (ammonium chloride) and metal ion concentration (zinc ion). In this work, a 2^3- full factorial Central Composite Design was employed combining with Response Surface Methodology (RSM) to optimize the process parameters for the degradation of phenol by *P.aeruginosa* (NCIM 2074). It was shown that a second order polynomial regression model could properly interpret the experimental data with an R^2 value of 0.9669 and an F-value of 32.52295 based on which the maximum degradation of phenol was estimated up to 80.45% within the range examined.

Keywords: Biodegradation, phenol, *Pseudomonas aeruginosa*, Central Composite Design.

INTRODUCTION

Phenols, i.e hydroxyl compounds of aromatic hydrocarbons, and its derivatives are widely used as raw materials in many petrochemical industries, pharmaceutical, pulp, paper, tannery, coal refining industries[1], antiseptics and disinfectants, pesticides and paints [2-5]. Thus, phenol is generally present in wastewater coming from these industries [6]. It has been found to affect the aquatic life, causing ecological imbalance. It is lethal to fish even at relatively low concentrations of 5-25 mg/l [7]. Phenol also imparts objectionable taste to municipal drinking water at far lower concentrations. Hence, phenol containing effluents have to be properly treated prior to discharge. As a consequence, the Environmental Protection Agency (EPA) has set a water purification standard of less than 1.0 ppb of phenols in surface waters. Therefore, the development of methods for the removal of phenols from industrial wastewater has generated significant interest.

Conventional methods of treatment for phenolic wastes have been largely chemical or physical such as chemical oxidation, solvent extraction and adsorption, but these processes have led to secondary effluent problems. Besides these methods, biological treatment i.e Biodegradation is preferred. Biodegradation is versatile, inexpensive and can potentially turn a toxic material into harmless products. If properly designed and operated, biological

process can realize total oxidation of organic matter so that there can be no sludges that must be eradicated as a result of treatment [8]. Moreover the use of pure cultures microorganisms, especially adapted to metabolize the contaminant, can be envisaged as an attractive alternative [9-13].

The optimization of growth conditions for phenol degradation is of primary importance in the development of the bioprocess. In general, optimization studies involving the one-factor at a time approach are not tedious, but tend to overlook the effects of interacting factors and might lead to misinterpretations of the results. On the other hand, statistical planned experiments effectively solve such problems; minimize the error in determining the effect of parameters and the results are achieved in an economical manner [14].

Response Surface Methodology, which is supported by software, is an empirical modelization technique derives for the evaluation of the relationship of a set of controlled experimental factors and observed results. It requires a prior knowledge of the processes to achieve statistical model. Basically, this optimization process involves three major steps: estimating the coefficients in a mathematical model, predicting the response and checking the adequacy of the model [8, 15-16].

In this study, phenol biodegradation was investigated in a batch reactor using *Pseudomonas aeruginosa* (NCIM 2074). Various environmental factors like carbon source (glucose, maltose, xylose, fructose, sucrose), inorganic nitrogen source (ammonium chloride, ammonium nitrate, ammonium tartrate, ammonium acetate), organic nitrogen source (peptone, tryptone, malt extract, urea) and metal ion concentration (iron, cadmium, copper, zinc) were studied. The effects of various process factors on the phenol degradation were discussed based on response surface methodology.

Response Surface Methodology

Response surface methodology is the most widely used statistical technique for bioprocess optimization. Response surface experiments identify the response of a system as a function of explanatory variables. It is often used to determine the optimal response for specific range of variable conditions. The interaction among the possible influencing parameters can be evaluated with limited number of experiments.

Response surface methodology is used to determine the optimal response of phenol degradation using *P.aeruginosa* (NICM 2074) considering various process parameters like carbon source, organic nitrogen source, inorganic nitrogen source, metal ion concentration.

For statistical calculations the variable x_i was coded X_i according to equation (1):

$$\mathbf{X_i = (x_i - x_0)/\Delta\ x\ \ i = 1,2,3,\ldots,k} \qquad \mathbf{(1)}$$

Where X_i is coded (dimensionless) value of the variable x_i, x_0 is the value of x_i at the centre point, Δx is the step change.

The system was stated by the following second-order, polynomial equation[16]:

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X^2{}_i + \sum b_{ij} X_i X_j \quad (2)$$

where Y is the predicted response, b_0 the offset term, b_i the linear effect, b_{ii} the square effect, and b_{ij} the interaction effect. The experimental variables glucose concentration, inorganic nitrogen source and metal ion concentration are the critical variables and designated as X_1, X_2, and X_3 respectively (Table 1). The low, middle, and high levels of each variable (equally spaced) are designated as -1, 0 and +1 respectively.

The mathematical relationship of the response Y and these four variables can be approximated by the quadratic (second-degree) polynomial equation:

$$\mathbf{Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1{}^2 + \ \ b_{22}X_2{}^2 + b_{33}X_3{}^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3} \qquad \mathbf{(3)}$$

This design is preferred because relatively few combinations of the variables are adequate to estimate potentially complex response function. The total 20 experiments are needed to calculate 9 coefficients of the second-order polynomial regression model. This model contains one block term, three linear terms, three quadratic terms, and two interaction

terms, which is applied by using design of experiments for phenol degradation.

MATERIALS AND METHODS

Materials

Phenol (99% purity), 4-amino antipyrine, and other chemicals were offered from Merck Co. The microorganism *P.aeruginosa* (NCIM 2074) was purchased from NCL Pune. The microorganism was stored at 4°C in a medium containing beef extract: 3.0 g l^{-1}; peptone: 5.0 g l^{-1}; sodium chloride: 5.0 g l^{-1} and agar: 20.0 g l^{-1}. The medium was adjusted to pH 7.2 by adding acid or base accordingly.

Experimental Procedures

The experimental range was optimized to maximize phenol degradation with the Central Composite Design method considering the solution carbon source (0.5,1.0,1.5,2.0,2.5 g/l) inorganic nitrogen (0.1,0.2,0.3,0.4 g/l), organic nitrogen source (0.2,0.4,0.6,0.8,1.0 g/l) and metal ion concentration (0.01,0.02,0.03,0.04,0.05 g/l). Experiments were carried out in conical flasks containing mineral medium, in a shaker and pure *P.aeruginosa* (NCIM 2074) was inoculated.

Estimation of Phenol

The concentration of phenol undegraded in the solution was determined by a UV-vis spectrophotometer using 4-amino antipyrine as a color reagent [17].

RESULT AND DISCUSSION

Optimization of nutritional parameters

Effect of carbon source

Microorganisms acquire nutrients, electrons and energy from their environments to support growth. Biodegradation of organic substrates provide microorganisms with energy and building materials that are used for growth of new cells, cell maintenance and co-metabolism of other less degradable substances [18].

In general, microorganisms grow mostly in a medium supplemented with additional substrates [19]. Hence, growth could be manipulated by addition of two or more nutrients simultaneously [20-22]. If a microbial population is grown on mixed substrates present in the medium, the microbes consume only one, or both the substrates. Consequently, several utilization patterns can be observed. In mixed substrates, individual substrates can have synergistic, antagonistic or no effect on one another, resulting in a growth rate that is higher, lower or the same than if the substrates are present individually [23-24].

Hence, different carbon sources were selected for two reasons. First, phenol is a toxic compound representing wastes of industrial origin. Second, these conventional carbon sources are non-toxic, a common substrate which can represent wastes of urban or agricultural origin. In this study, the effect of six different carbon sources namely glucose, galactose, maltose, xylose, fructose and sucrose in the range of 0.5-2.5 g/l on the degradation of phenol were studied and shown in Figure 1. From Figure 1, it can be observed that glucose is the best carbon source among all for phenol degradation. Phenol degradation increased up to a glucose concentration of 0.5 g/l and thereafter it decreased and the degradation was almost inhibited at a concentration of 2.5 g/l. This may be due to catabolite repression by glucose as reported by Papanastasiou (1982)[25] i.e the presence of glucose could inhibit utilization of the target substrate. Satsangee and Ghosh 1990 [26] have also reported that glucose interferes with phenol uptake. This result coincides with Kar et al. 1996 [27] who reported that phenol degradation was completely inhibited when glucose concentration was at 2 g/l. Hence 0.5 g/l glucose concentration was considered to be the optimum carbon source.

Effect of nitrogen source

Nitrogen is the next most important nutrient for the phenol degradation. Nitrogen is used from different sources either from inorganic or from organic.

Effect of inorganic nitrogen source

The effect of four inorganic nitrogen sources namely ammonium chloride, ammonium nitrate, ammonium tartrate, and ammonium acetate in the range of 0.1-0.4 g/l on the degradation of phenol were studied and shown in Figure 2. From Figure 2, it was observed that at a concentration of 0.2 g/l of ammonium chloride, the phenol degradation was increased and further increase in the ammonium chloride concentration, a detrimental effect was observed on phenol degradation. Hence, the optimum concentration of ammonium chloride observed was 0.2 g/l and the phenol degradation was 73.28%. The enhanced rate of phenol degradation at less than 0.2 g/l

ammonium chloride can be attributed to the attenuation of phenol toxicity by ammonium chloride and the increase in cell mass formed as a result of the additional nitrogen source. This is in good agreement with Premalatha and Suseela Rajakumar 1994 [28] who reported that ammonium chloride at a concentration of 0.175 g/l was the best nitrogen source for pentachlorophenol degradation, giving 100% degradation by day 5. Pentachlorophenol degradation by a mixed bacterial population was also enhanced by ammonium salts [29].

Effect of organic nitrogen source

The effect of four organic nitrogen sources viz peptone, tryptone, malt extract and urea in the range of 0.25-1.00 g/l was studied and the results were shown in Figure 3. From Figure 3, it was observed that phenol degradation increased up to a concentration of 0.25 g/l and with further increase in the concentration of peptone, phenol degradation started decreasing. Hence, among the organic nitrogen sources tested, peptone at a concentration of 0.25 g/l was the best source for maximum phenol degradation. The percentage phenol degradation with the addition of optimal concentration of peptone was 74.67%. The present study indicated that peptone at low concentrations influences the rate of phenol degradation.

This observation was in good agreement with that of Kotresha and Vidyasagar 2008 [30] who reported that at a concentration of 0.25 g/l, peptone enhanced maximum phenol degradation using *P.aeruginosa* MTCC 4996. Similar results were reported by Lob and Tar 2000 [31].

Effect of metal ions

The tolerance of different strains to metals varies widely and it is necessary to determine the optimum concentration to avoid the inhibitory effects caused when these cations are present in toxic concentrations. The present study investigates the effect of four different metal sources viz iron, cadmium, copper and zinc in the range of 0.01- 0.05 g/l on phenol degradation by *P.aeruginosa* (NCIM 2074). From Figure 4, it was observed that zinc degraded maximum phenol when compared to iron, cadmium and copper.

It may be observed that from Figure 4, that phenol degradation increased up to a concentration of 0.02 g/l and further increase in zinc concentration had a detrimental effect on phenol degradation. Hence, the optimum concentration of zinc for phenol degradation by *P.aeruginosa* (NCIM 2074) was found to be 0.02 g/l. This result is in agreement with Kotresha and Vidyasagar 2008 [30] who reported that maximum degradation of phenol by *P.aeruginosa* MTCC 4996 was possible in the presence of 2.0 mM zinc. This may be due to the fact that microbes display a large range of tolerance and resistance to heavy metals [32]. Hughes and Poole (1989) and Sterritt and Lester (1980) [33-34] also reported that addition of certain metal ions at low concentration enhances the degradation rate.

Evaluation of experimental results with CCD

The three nutritional factors which influence the phenol degradation highly are glucose, ammonium chloride and zinc ion (Zn^{2+}). Hence these three factors are considered as major nutritional parameters to optimize. The suitable levels of these parameters were determined using CCD. Experiments were carried out as per the design, and the percentage phenol degradation with 20 experimental runs and with different combinations of glucose concentration, ammonium chloride concentration and metal ion concentration (Zn^{2+}) were estimated.

The experimental design matrix was given in Table 1.

By applying multiple regression analysis on the experimental data, the following second order polynomial equation was found to represent the percentage phenol degradation adequately.

$$Y = 58.7 + 29.8X_1 + 60.8X_2 + 848.9X_3 - 62.2X_1^2 - 297.8X_2^2 - 33742.3X_3^2 + 113.5X_1X_2 + 563.0X_3X_1 \quad (4)$$

The predicted values of percentage phenol degradation using the above equation were given in Table 2 along with experimental values. The coefficients of the regression model (Eq. 4) calculated were listed in Table 3, in which they contain three linear, three quadratic and three interaction terms and one block term. The significance of each coefficient was determined by student's *t*-test and *p*-values, which were listed in Table 3. The

larger the magnitude of the *t*-value and smaller the *p*-value, the more significant is the corresponding coefficient. This implies that the first order and second order main effects of glucose concentration, ammonium chloride concentration and metal ion concentration (Zn^{2+}) were highly significant as is evident from their respective *p*-values. They were more significant at the second order. This indicates that they can act as limiting nutrients and small variations in their concentration will alter either growth rate or product formation rate or both to a considerable extent. The interaction effect of glucose concentration × ammonium chloride and metal ion concentration (Zn^{2+}) × glucose concentration were found to be significant ($p \leq 0.05$). The interaction term i.e. ammonium chloride × metal ion concentration (Zn^{2+}) was found to be insignificant (Table 3).

The parity plot (Figure 5) showed a satisfactory correlation between the experimental and predicted values (obtained from Eq. (4) of percentage phenol degradation, wherein, the points cluster around the diagonal line indicated the optimal fit of the model, since the deviation between the experimental and predicted values was minimal.

The results of the second order response surface model fitting in the form of ANOVA were given in Table 4. It is required to test the significance and adequacy of the model. The Fisher variance ratio, the *F*-value ($= S^2_r / S^2_e$), is a statistically valid measure of how well the factors describe the variation in the data about its mean. The greater the *F*-value is from unity, the more certain it is that the factors explain adequately the variation in the data about its mean, and the estimated factor effects are real. The ANOVA of the regression model demonstrates that the model is highly significant, as is evident from the Fisher's *F*-test (F_{model} = 32.52295) and a very low probability value ($P_{model} > F$=0.000003).

The goodness of the fit of the model was checked by the determination coefficient (R^2). The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental variables and their interactions. The R^2 value is always between 0 and 1. The closer the R^2 value is to 1, the stronger the model is and the better it predicts the response. In this case, the value of the determination coefficient (R^2 = 0. 9669) indicates that 96.69 % of the variability in the response could be explained by the model. In addition, the value of the adjusted determination coefficient (Adj R^2 = 0.9372) is also very high to advocating a high significance of the model. The predicted and experimental percentage phenol degradation at the optimum levels of nutritional conditions was also determined by using Eq. (4).

Figures 6-8 represents the isoresponse contour and surface plots for the optimization of nutritional conditions of phenol degradation. The effect of the glucose concentration and ammonium chloride concentration on the percentage phenol degradation was shown in Figure 6. An increase in the ammonium chloride concentration with glucose concentration up to the optimum point increased the percentage phenol degradation to a maximum level and a further increase in the ammonium chloride concentration with glucose concentration, the trend is reversed.

The interaction effect of the metal ion concentration (Zn^{2+}) and ammonium chloride concentration on the percentage phenol degradation shown in Figure 7 clearly indicates a proper combination for degradation of phenol. An increase in the ammonium chloride concentration with metal ion concentration (Zn^{2+}) increased the phenol degradation gradually but at a higher ammonium chloride concentration and metal ion concentration (Zn^{2+}) the trend is reversed. The optimum value for maximum phenol degradation lies near the centre point of the ammonium chloride concentration and metal ion concentration (Zn^{2+}).

A similar effect on the response was observed for the glucose concentration at any level of the metal ion concentration (Zn^{2+}). An increase in the glucose concentration with metal ion concentration (Zn^{2+}) up to the optimum point increased the percentage phenol degradation to a maximum level and a further increase in the glucose concentration with metal ion concentration (Zn^{2+}) decreased the phenol degradation as shown in Figure 8.

Therefore, an optimum was observed near the central value of glucose concentration, ammonium chloride concentration and metal ion concentration (Zn^{2+}). The optimum conditions for maximum phenol degradation were obtained at a glucose concentration of 0.5149 g/l, ammonium chloride concentration of 0.2118 g/l and metal ion concentration (Zn^{2+}) of 0.0181 g/l. A maximum percentage phenol degradation of 80.45 was obtained at

these optimum parameters. The experimental and predicted values of phenol degradation at optimum conditions of degradation were also determined (Table 5). At the optimum conditions of physical and nutritional parameters, maximum percentage phenol degradation of 80.45 was obtained.

CONCLUSION

Contamination of the environment with hazardous and toxic chemicals is one of the major problems faced by industrialized nations today. *P.aeruginosa* (NCIM 2074) was studied of its potential for degrading phenol. The effects of nutritional parameters of carbon source (glucose, maltose, xylose, fructose, sucrose), inorganic nitrogen source (ammonium chloride, ammonium nitrate, ammonium tartrate, ammonium acetate), organic nitrogen source (peptone, tryptone, malt extract, urea) and metal ion concentration (iron, cadmium, copper, zinc) on phenol removal were elucidated batch-wise. Utilization of (0.5,1.0,1.5,2.0,2.5 g/l), ammonium chloride (0.1,0.2,0.3,0.4 g/l), peptone (0.2,0.4,0.6,0.8,1.0 g/l), Zn^{+2} (0.01,0.02,0.03,0.04,0.05 g/l) concentration were, however, quick and normally preceded phenol degradation. At these optimized conditions, percentage of phenol removal was 76.34. The interactions among these nutritional parameters were studied using RSM. The levels of these parameters were determined using CCD. The optimum conditions for maximum phenol degradation were obtained at 0.5149 g/l of glucose concentration, 0.2118 g/l of ammonium chloride concentration, 0.0181 g/l of Zn^{+2} concentrations. At these optimum conditions, maximum phenol degradation obtained was 80.45.

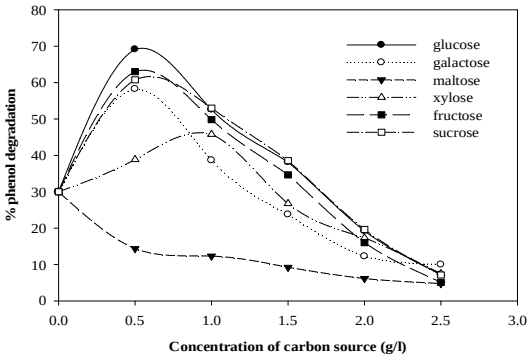

Fig. 1: Effect of carbon source on the biodegradation of phenol by *P.aeruginosa* (NCIM 2074)

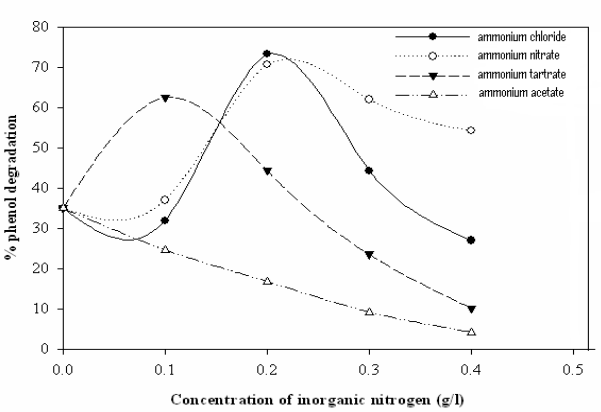

Fig. 2: Effect of inorganic nitrogen source on the biodegradation of phenol by *P.aeruginosa* (NCIM 2074)

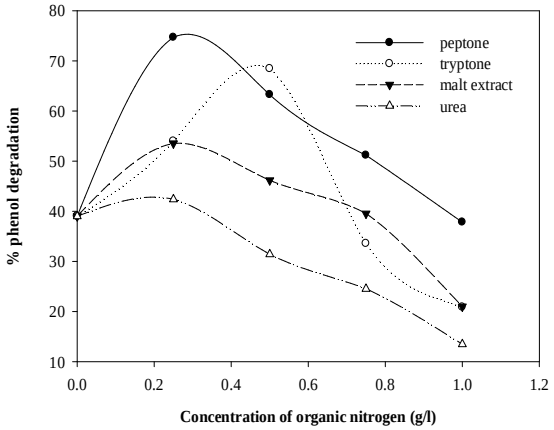

Fig. 3: Effect of organic nitrogen source on the biodegradation of phenol by *P.aeruginosa* (NCIM 2074)

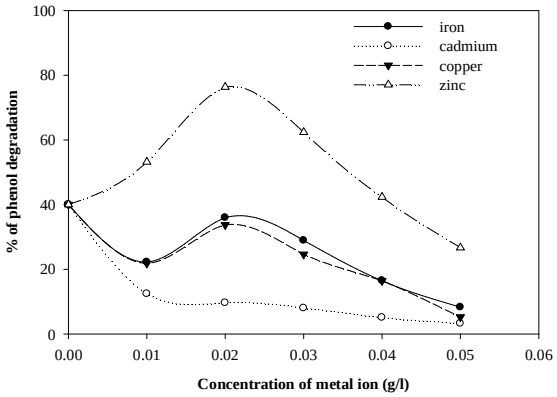

Fig. 4: Effect of metal ion concentration on the biodegradation of phenol by *P.aeruginosa* (NCIM 2074)

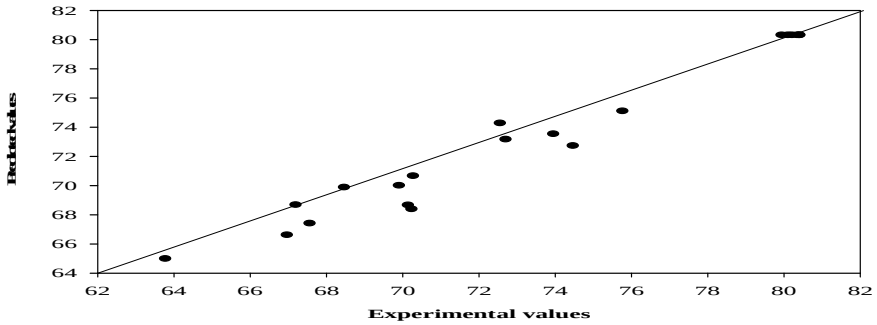

Fig. 5: Parity plot showing the distribution of predicted vs. experimental values of percentage phenol degradation for the nutritional parameters

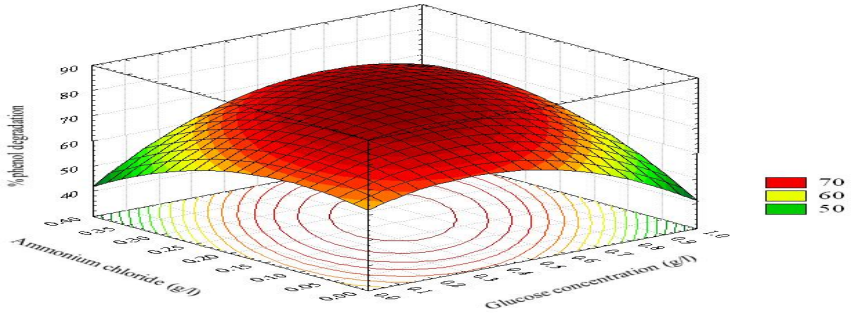

Fig. 6: Response and contour plot of glucose concentration vs. ammonium chloride on percentage phenol degradation (metal ion concentration (Zn^{2+}) was keptconstant at 0.02 g/l)

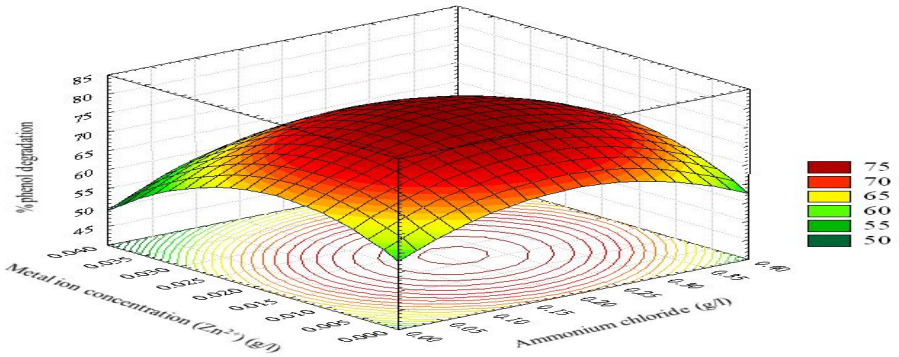

Fig. 7: Response and contour plot of metal ion concentration (Zn^{2+}) vs. ammonium chloride on percentage phenol degradation (glucose concentration was kept constant at 0.5 g/l)

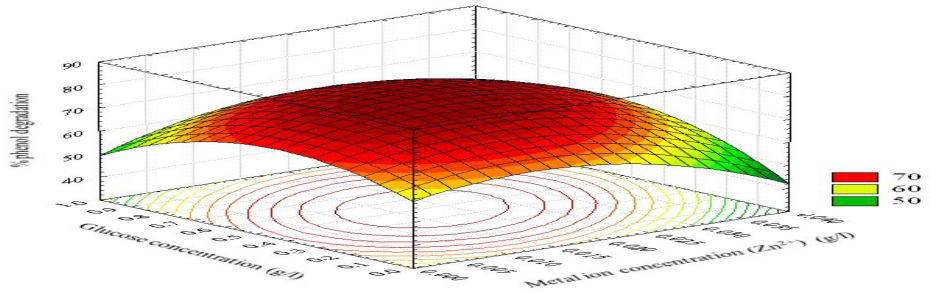

Fig. 8: Response and contour plot of metal ion concentration (Zn^{2+}) vs. glucose concentration on percentage phenol degradation (ammonium chloride was kept constant at 0.2 g/l)

Table 1: CCD matrix employed for the optimization of nutritional parameters for the degradation of phenol by *P.aeruginosa* (NCIM 2074)

Run No.	Coded & real values of glucose (X_1)	Coded & real values of ammonium chloride (X_2)	Coded & real values of zinc ion (X_3)
1.	–1(0.25)	–1(0.1)	–1(0.01)
2.	–1(0.25)	–1(0.1)	1(0.03)
3.	–1(0.25)	1(0.3)	–1(0.01)
4.	–1(0.25)	1(0.3)	1(0.03)
5.	1(0.75)	–1(0.1)	–1(0.01)
6.	1(0.75)	–1(0.1)	1(0.03)
7.	1(0.75)	1(0.3)	–1(0.01)
8.	1(0.75)	1(0.3)	1(0.03)
9.	–1.682(0.08)	0(0.2)	0(0.02)
10.	1.682(0.92)	0(0.2)	0(0.02)
11.	0(0.5)	–1.682(0.03)	0(0.02)
12.	0(0.5)	1.682(0.37)	0(0.02)
13.	0(0.5)	0(0.2)	–1.682(0.0032)
14.	0(0.5)	0(0.2)	1.682(0.0368)
15.	0(0.5)	0(0.2)	0(0.02)
16.	0(0.5)	0(0.2)	0(0.02)
17.	0(0.5)	0(0.2)	0(0.02)
18.	0(0.5)	0(0.2)	0(0.02)
19.	0(0.5)	0(0.2)	0(0.02)
20.	0(0.5)	0(0.2)	0(0.02)

Table 2: CCD matrix showing real values of nutritional parameters along with the experimental and predicted values of percentage phenol degradation

S. No.	X_1	X_2	X_3	Percentage phenol degradation	
				Experimental	Predicted
1.	0.25	0.1	0.01	75.78	75.09722
2.	0.25	0.1	0.03	67.21	68.66724
3.	0.25	0.3	0.01	68.48	69.86891
4.	0.25	0.3	0.03	63.79	64.97893
5.	0.75	0.1	0.01	67.58	67.40412
6.	0.75	0.1	0.03	66.98	66.60414
7.	0.75	0.3	0.01	73.97	73.52581
8.	0.75	0.3	0.03	72.57	74.26583
9.	0.08	0.2	0.02	70.16	68.65274
10.	0.92	0.2	0.02	69.92	69.99154
11.	0.50	0.03	0.02	70.29	70.64974
12.	0.50	0.37	0.02	74.48	72.71812
13.	0.50	0.2	0.0032	72.72	73.15692
14.	0.50	0.2	0.0368	70.25	68.37735
15.	0.50	0.2	0.02	80.23	80.30023
16.	0.50	0.2	0.02	80.42	80.30023
17.	0.50	0.2	0.02	80.12	80.30023
18.	0.50	0.2	0.02	79.96	80.30023
19.	0.50	0.2	0.02	80.37	80.30023
20.	0.50	0.2	0.02	80.42	80.30023

Table 3: Coefficients, t-statistics and significance of the model for the nutritional parameters

Term	Coefficient	Value	Standard error of coefficient	*t*- value	*p*-value
Constant	b_0	58.7	4.235	13.8571	0.000000*
glucose concentration	b_1	29.8	8.064	3.6972	0.004127*
ammonium chloride	b_2	60.8	19.961	3.0438	0.012382*
metal ion concentration (Zn^{2+})	b_3	848.9	201.592	4.2112	0.001797*
glucose concentration × glucose concentration	b_{11}	-62.2	5.756	-10.8028	0.000001*
ammonium chloride × ammonium chloride	b_{22}	-297.8	35.288	-8.4394	0.000007*
metal ion concentration (Zn^{2+})× metal ion concentration (Zn^{2+})	b_{33}	-33742.3	3597.403	-9.3796	0.000003*
glucose concentration × ammonium chloride	b_{12}	113.5	19.273	5.8891	0.000153*
ammonium chloride × metal ion concentration (Zn^{2+})	b_{23}	385.0	481.821	0.7991	0.442825
metal ion concentration (Zn^{2+})× glucose concentration	b_{31}	563.0	192.729	2.9212	0.015269*
* Significant ($p \leq 0.05$)					

Table 4: ANOVA for the entire quadratic model for the nutritional parameters

Source of variation	Sum of squares (SS)	Degree of freedom (DF)	Mean squares (MS)	F-value	Probe > F
Regression	543.6190	9	60.40211	32.52295	0.000003
Residual	18.5721	10	1.85721		
Total	562.1911				
R^2= 0.9669 Adjusted R^2=0.9372					

Table 5: Optimum values of nutritional parameters: experimental and predicted values of the percentage phenol degradation

Variables	Optimum values	Optimum percentage phenol degradation	
		Experimental	Predicted
Eq. (4.2)		80.45	80.47
glucose concentration (g/l), (X_1)	0.5149		
ammonium chloride (g/l), (X_2)	0.2118		
metal ion concentration (Zn^{2+}) (g/l),(X_3)	0.0181		

REFERENCES

1. Paula M Van schei and Young LY. Isolation and characterization of phenol degrading denitrifying bacteria. Applied Environmental Microbiology. 1998; 64(7):2432-2438.
2. TARC Thailand Accident Research Center. Phenol and pollution. 1989; TARC, 115.
3. ANZECC Australian and New Zealand Environment and Conservation Council. Australian water quality guidelines for fresh and marine water.1992.
4. WHO. IPCS Environmental health criteria for phenol first draft prepared by Montizan GK, Published by WHO. 1994. Printed in Finland.
5. ATSDR. Agency for Toxic Substances and Disease Registry. Toxicological profile for phenol, Atlanta, Coorgia. 2005:111.
6. Marrot B, Barrios-Martinez A, Moulin P and Roche N. Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. Biochemical Engineering Journal. 2006;30(2):174–183.
7. Saha NC, Bhunia F and Kaviraj A. Toxicity of phenol to fish and aquatic ecosystems. Bull Environ Contam Toxicol. 1999;63:195–971.

8. Annadurai G, Ling LY and Lee JF. Statistical optimization of medium components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*. Journal of Hazardous Materials. 2008;151:171-178.
9. Allsop PJ, Chisti Y, Moo-Young M and Sullivan GR. Dynamics of phenol degradation by *Pseudomonas putida*. Biotechnology and Bioengineering. 1993;41:572-580.
10. Wang SJ and Loh KC. Modeling the role of metabolic intermediates in kinetics of phenol biodegradation. Enzyme and Microbial Technology. 1999;25(3-5):177-184.
11. Jiang Y, Wen J, Jia X, Caiyan Q and Hu Z. Mutation of *Candida tropicalis* by irradiation with a He-Ne laser to increase its ability to degrade phenol. Applied and Environmental Microbiology. 2007;73(1):226-231.
12. Yang CF and Lee CM. Enrichment, isolation, and characterization of phenol-degrading *Pseudomonas resinovorans* strain P-1 and *Brevibacillus* sp. strain P-6. International Biodeterioration and Biodegradation. 2007;59:206-210.
13. Wei G, Yu J, Zhu Y, Chen W and Wang L. Characterization of phenol degradation by *Rhizobium* sp. CCNWTB 701 isolated from *Astragalus chrysopteru* in mining tailing region. Journal of Hazardous Materials. 2007;151(1):111-117.
14. Abdel-Fattah YR, El-Helow ER, Ghanem KM and Lotfy WA. Application of factorial designs for optimization of avicelase production by a thermophilic *Geobacillus* isolate. Journal of Microbiology. 2007;2:13-23.
15. Box GEP and Draper NR. A basic for the selection of a response surface design. Journal of American Statistical Association. 1959;54(287):622-654.
16. Box GEP and Behnken DW. Some new three-level design for the study of quatitative variables. Technometrics. 1960;2:455-475.
17. Clesseri CS, Greenberg AB and Trussel RR. Standard methods for the determination of water and wastewater. APHA, Washington, DC. 1985:5.48-5.53.
18. Cornelissen G, and Sijm DT An energy budget model for the biodegradation and co-metabolism of organic substances. Chemosphere. 1996;33(5):817-830.
19. Harder W, Dijkhuizen I and Postgate JR. Strategies of mixed substrate utilization in microorganisms. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 1982;297(1088):459-480.
20. Rutgers M, Balk PA and van Dam K. Quantification of multiple-substrate controlled growth-simultaneous ammonium and glucose limitation in chemostat cultures of *Klebsiella pneumoniae.* Archives of Microbiology. 1990;153(5):478-484.
21. Egli T. On multiple-nutrient-limited growth of microorganisms, with special reference to dual limitation by carbon and nitrogen substrates. Antonie van Leeuwenhoek. 1991;60(3-4):225-234.
22. Egli T. The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrate. Advances in Microbial Ecology. 1995;14:305-386.
23. Meyer JS, Marcus MD and Bergman HL. Inhibitory interactions of aromatic organics during microbial degradation. Environmental Toxicology and Chemistry. 1984;3(4):583-587.
24. Saez PB and Rittmann BE. Biodegradation kinetics of 4-chlorophenol, an inhibitory co-metabolite. Research Journal of the Water Pollution Control Federation. 1991;63(6):838-847.
25. Papanastasiou AC and Maiwer WJ. Kinetics of biodegradation of 2,4-dichlorophenoxyacetate in the presence of glucose. Biotechnology and Bioengineering. 1982;24(9):2001-2011.
26. Satsangee R and Ghosh P. Anaerobic degradation of phenol using an acclimated mixed culture. Applied Microbiology and Biotechnology. 1990;34(1):127-130.

27. Kar S, Swaminathan T and Baradarajan A. Studies on biodegradation of a mixture of toxic and nontoxic pollutant using *Arthrobacter* species. Bioprocess Engineering. 1996;15(4):195-199.
28. Premalatha A and Suseela Rajakumar G. Pentachlorophenol degradation by *Pseudomonas aeruginosa*. World Journal of Microbiology and Biotechnology. 1994;10(3):334- 337.
29. Valo R, Apajalahti J and Salkinoja Salonen M. Studies on the physiology of microbial degradation of pentachlorophenol. Applied Microbiology and Biotechnology. 1985;21(5):313-319.
30. Kotresha D and Vidyasagar GM. Isolation and characterization of phenol-degrading *Pseudomonas aeruginosa* MTCC 4996. World Journal of Microbiology and Biotechnology. 2008;24(4):541-547.
31. Lob KC and Tar PPC. Effect of additional carbon sources on biodegradation of phenol. Bulletin of Environmental Contamination and Toxicology. 2000;64(6):756-763.
32. Trevors JT, Oddie KM and Belliveau BH. Metal resistance in bacteria. FEMS Microbiology Reviews. 1985;32(1):39-54.
33. Hughes MN and Poole RK. Metal toxicity. In: Hughes MN, Poole RK (eds) Metals and Microorganisms, Chapman and Hall, New York. 1989:252-302.
34. Sterritt RM and Lester JN. Interactions of heavy metals with bacteria. Science Total Environment. 1980;14:5-17.