www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.12, pp 4934-4939, October 2014

CBSE-2014 [2nd and 3rd April 2014]
Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Statistical Evaluation of Biodegradation of O-Cresol using *Aspergillus fumigates*

P.Balamurugan* and B.Preetha

Department of Chemical Engineering, Annamalai University, Annamalai Nagar, India

***Corres.author: bala_790315@rediffmail.com**

Abstract : Biodegradation is widely used for removal of toxic organic contaminants from aqueous streams. Owing to the hazardous or otherwise undesirable characteristics of phenolic compounds in particular, their presence in wastewater from municipal and industrial discharge is one of the most important environmental issue. Response surface methodology is a widely used technique for modelling and optimization of the biodegradation treatment processes of water and wastewater. This methodology not only estimates linear, interaction and quadratic effects of the factors on the response, but also provides a prediction model for the response at the range of the variables studied and the optimum conditions to achieve the highest performance. In this work, statistical design was applied for the optimization of process parameters for the biodegradation of O-Cresol using *Aspergillus fumigates* (MTCC No.343) in a batch reactor. The effects of process parameters such as initial cresol concentration (100 – 500 ppm), pH (5-7), and temperature (30-45^{O}C) on Removal Efficiency (RE) of Cresol was studied and optimized using Response Surface Methodology (RSM). Using Central Composite Design (CCD), 20 experiments were carried out for the three test variables. A second order polynomial regression model has been developed using the experimental data. It was found that the degrading potential of *Aspergillus fumigates* was strongly affected by the variations in pH, temperature and initial cresol concentration. From the results, the optimum condition for maximum RE of O-cresol wars found to be initial Cresol concentration - 150ppm, pH – 6.6 and temperature- 31^{o}C . At these optimized conditions, the removal efficiency of Cresol was found to be 80.41%. A high R^2 value of greater than 0.9 indicates the fitness of the model to predict the experimental data.
Keywords: O-Cresol; Biodegradation; Response Surface Methodology; Aspergillus fumigates; Central Composite Design.

Introduction

An enormous amount of organic compounds are released into the waste stream of various industries. Among them, the cresols are highly toxic compounds. *O*-cresol is an isomeric phenol with methyl substituent in the orto position relative to the hydroxyl group. *O*-cresol has a wide variety of usages comprising as disinfectants, fumigants in dyes and odors, in photographic developers, in pesticides which are used extensively in agricultural methods, etc.[1]. Several animal studies suggest that cresols may promote tumor growth. United States Environmental Protection Agency (USEPA) has classified cresols within the C: Possible human carcinogens [2]. In addition to being highly toxic and potentially carcinogenic, according to the Agency for Toxic Substances and Disease Registry (ATSDR) cresols cause, even at very low concentrations, adverse effects on the nervous system, cardiovascular system, lungs, kidney and liver resulting in central nervous system depression[3].

Early pioneer studies were based on physicochemical absorption of organic pollutants [4]. Biodegradation has rapidly become the most effective modality for complete mineralization of organic pollutants. In the natural environment, the microorganisms perform a major role in the biodegradation of toxic chemicals. Phenol is the most widely focused compound in biodegradation studies , even though a few of them consider cresol derivatives as a mixture of phenolic pollutants [5-7]. Several microorganisms utilize cresol as a sole carbon source, despite of its toxicity [8]. These microorganisms may be fungi or algae, but the most practicable one is bacteria; especially Pseudomonas genus, because of its growth rate and efficiency, most studies on degradation of phenolic compounds have been carried out using bacteria [9-10].

Although the biodegradation of phenolic wastes has been extensively studied with various types of microorganisms [11-12], it is hard to find those in which present data is sufficient to illuminate the kinetic properties of potential microorganisms [13] and that are based on *o*-cresol as a sole carbon source[14-15]. In this study, we tried to demonstrate that *o*-cresol, one of the substantially toxic compounds, can be exploited by *Aspergillus fumigatus* by response surface methodology.

Materials and Methods

Materials

The microorganisms Aspergillus fumigatus *(MTCC No.343)* was purchased from microbial type culture collection center, Chandigarh. The microorganism was stored at 4°C in a medium containing Czapek concentrated: 10 ml l^{-1}, K_2HPO_4:1.0 g l^{-1}, Yeast extract: 5.0 g l^{-1}; glucose: 30.0 g l^{-1}. The medium was adjusted to pH 7.3 by adding acid or base accordingly.

Experimental Design and Procedure

Table 1 Ranges of the independent variables used in RSM

Variables	Levels					
	Code	-1.68	-1	0	+1	+1.68
Initial Cresol concentration	X1	66	100	150	200	234
pH	X2	5.2	5.5	6	6.5	6.8
Temperature	X3	27.3	30	34	38	40.1

Response surface methodology was used in this study. The experimental variables at different levels used for the removal of phenol using BBD were given in Table 1. A total of 30 runs were used to optimize the parameters. The experimental design was carried out using Design Expert 7.1.5 (Stat Ease, USA). BBD was used to identify the optimum operating condition in order to obtain maximum RE as response. The collection of experiments provides an effective means for optimization through these process variables. Besides, the design permits the estimation of all main and interaction effects. A second-degree quadratic polynomial is used to represent the function in the range of interest.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1, i<j}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \qquad (1)$$

where X_1, X_2, X_3, X_4, . . ., X_k are the input variables which affect the response Y and β_0, β_i, β_{ii} and β_{ij} are the constants. A second-order model was designed such that variance of *Y* is constant for all points equidistant from the center of the design.

Experimental Procedures

The experimental range was optimized to maximize cresol degradation with the Central Composite Design method considering the initial cresol concentration (100,200,300,400 and 500 PPM), pH

(5,5.5,6.6.5 and 7) and temperature (30, 33,36,39, 42 and 45) . Experiments were carried out in conical flasks containing mineral medium, in a shaker and pure Aspergillus fumigatuas was inoculated.

O-cresol determination

O-cresol determinations were performed using a spectrometric method employing 4-aminoantipyrine (4-AAP) as a colour reagent. The method is based on the reaction between *o*-cresol and 4-AAP in the presence of ferricyanide at pH 10 to form a coloured antipyrine dye. The absorbance of the dye was measured at 505 nm.

Result and Discussion

Optimization of process parameters for the cresol degradation by Aspergillus fumigates

Table 2 Central composite design (CCD) of factors in coded levels with removal efficiency of cresol as response

Run No.	X_1	X_2	X_3	Removal efficiency of cresol	
				Experimental	Predicted
1	0.00	1.68	0.00	26.44	31.3132
2	1.00	1.00	1.00	32.17	32.4731
3	1.00	-1.00	-1.00	27.07	38.2333
4	0.00	0.00	0.00	76.67	77.0491
5	0.00	0.00	-1.68	64.98	56.9243
6	0.00	0.00	0.00	80.41	77.0491
7	0.00	0.00	0.00	78.31	77.0491
8	0.00	0.00	0.00	74.70	77.0491
9	0.00	0.00	0.00	80.22	77.0491
10	-1.00	-1.00	-1.00	65.17	59.8754
11	1.00	-1.00	1.00	37.93	24.3228
12	0.00	0.00	0.00	73.23	77.0491
13	0.00	-1.68	0.00	37.94	40.1410
14	1.00	1.00	-1.00	43.96	38.9684
15	-1.00	1.00	1.00	30.95	35.4425
16	0.00	0.00	1.68	25.26	40.3899
17	-1.00	1.00	-1.00	32.60	41.2157
18	1.68	0.00	0.00	29.04	30.8775
19	-1.68	0.00	0.00	46.26	51.4856
20	-1.00	-1.00	1.00	56.09	35.4425

CCD is used to determine the optimum conditions for the removal efficiency of cresol using *Aspergillus fumigatus*. The range and levels of factors (initial cresol concentration, pH and temperature) are given in Table 1. Twenty experiments are performed at different combinations. The predicted and observed responses along with design matrix are presented in Table 2. The results are analyzed by ANOVA. The second order regression equation provides the removal efficiency of cresol as the function of initial cresol concentration, pH and temperature. This can be presented in terms of coded factors as:

$$Y = 77.10 -5.32 X_1 - 4.83X_2 - 5.75 X_3 -13 X_1^2 -14.69 X_2^2 - 10.36\ 44X_3^2 + 8.60 X_1X_2 + 1.22 X_1X_3 -1.90X_2X_3 \quad \ldots (2)$$

where Y is the removal efficiency of cresol (%), X_1, X_2 and X_3 are Initial cresol concentration, pH and temperature respectively.

ANOVA for the response surface is shown in Table 3. The model F-value of 14.32 implies that the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. Values greater than 0.1 indicate the model terms are not significant. In the present work, all the linear, interactive effects of X_1X_2 and square effects of X_1, X_2 and X_3 are significant for removal of cresol. The coefficient of determination (R^2) for removal of cresol is calculated as 0.9280, which is very close to 1 and can explain upto 92.80% variability of the response. The predicted R^2 value of 0.7640 is in reasonable agreement with the adjusted R^2 value of 0.8633. An adequate precision value greater than 4 is desirable. The adequate precision value of 10.043 indicates an adequate signal and suggests that the model can be used to navigate the design space.

Table 3 Analysis of Variance (ANOVA) for response surface quadratic model for the Removal of cresol

Source	Coefficient factor	Sum of squares	DF	F	P > F
Model	77.10	7824.699	9	14.32987	0.0001
X_1	-5.32	386.4631	1	6.369789	0.0302
X_2	--4.83	318.29	1	5.246141	0.0450
X_3	-5.75	450.3788	1	7.423264	0.0214
X_1* X_2	8.60	592.2261	1	9.76123	0.0108
X_1* X_3	1.22	11.99721	1	0.197741	0.6660
X_2* X_3	-1.90	28.95247	1	0.477202	0.5054
X_1* X_1	-13	2428.592	1	40.02871	< 0.0001
X_2* X_2	-14.69	3204.591	1	52.81893	< 0.0001
X_3* X_3	-10.36	1540.528	1	25.3914	0.0005
Residual		606.7126	10		
Lack of fit		563.8305	5	13.14839	0.0067
Pure Error		42.88209	5	14.32987	0.0001
Cor Total		8431.412	19	6.369789	0.0302

Std. Dev. – 7.79; R^2 - 92.80%; Mean – 50.97; Adj R^2 – 86.33%; C.V. % - 15.28; Pred R^2 - 76.40%; Adeq Precision - 10.043

Equation 2 can be used to predict the removal of cresol within the limits of the experimental factors. Fig. 1 shows that the actual response values agree well with the predicted response values.

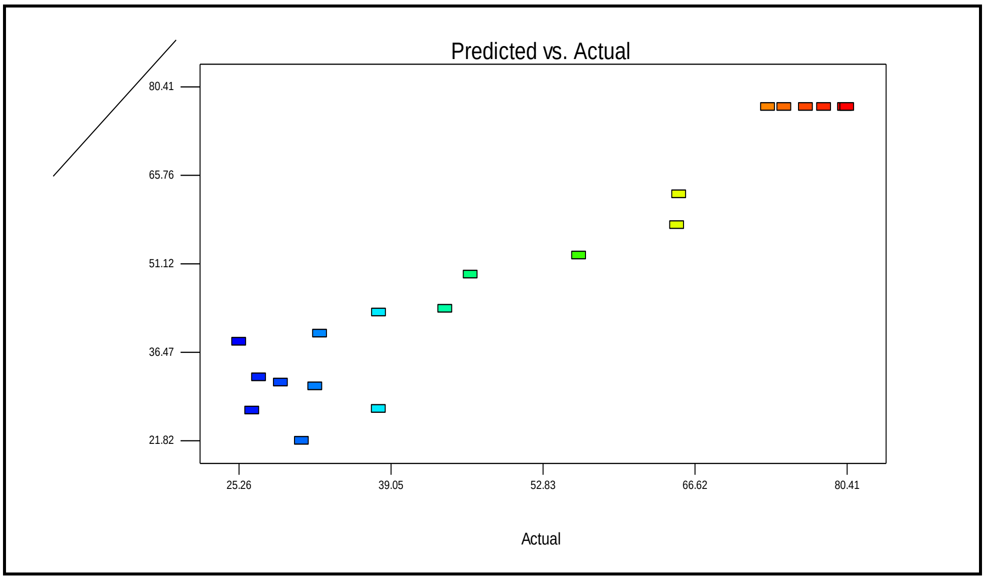

Fig. 1 Predicted response versus actual value of removal efficiency of cresol

The interactive effects of variables on removal of cresol are studied by plotting 3D surface curves against any two independent variables, while keeping the other variables at its central (0) level. The 3D curves of the calculated response (removal of cresol) and contour plots from the interactions between the variables are shown in Figs. 2 - 4. Fig. 2 shows the dependency of removal of cresol on initial cresol concentration and pH. The removal of cresol increases with increase in initial cresol concentration of upto 150 mg/l and thereafter removal of cresol decreases with further increase in initial cresol concentration. The same trend is observed in Fig. 3. Increase in pH resulted increase in removal of cresol upto 6.8. This is evident from Figs. 2 and 4. Figs.3 and 4 shows the dependency of removal of cresol on temperature. The effect of temperature on removal of cresol observed is similar to initial cresol concentration. The optimum conditions for the maximum removal of cresol are: initial cresol concentration – 150 mg/l, pH – 6.6 and Temperature -31°C.

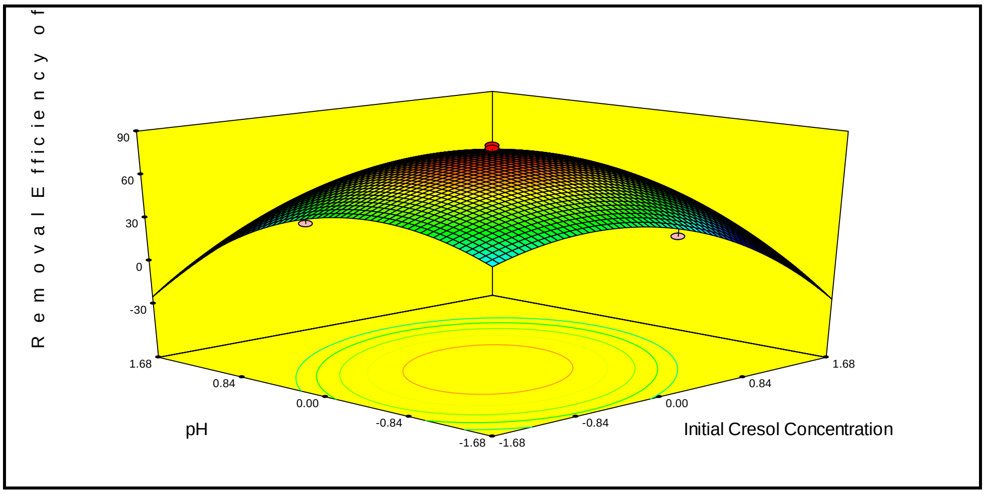

Fig. 2 3D plot showing the effect of initial cresol concentration and pH on Removal efficiency of cresol

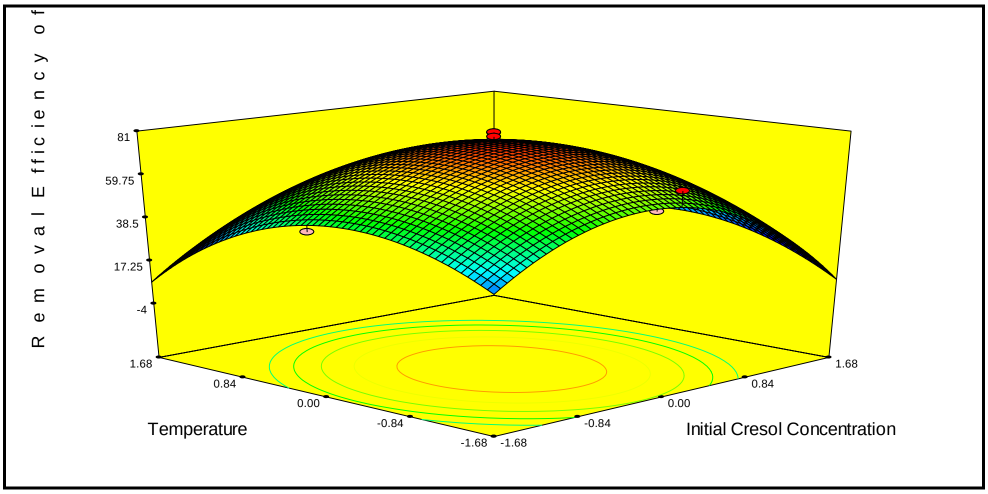

Fig. 3 3D plot showing the effect of initial cresol concentration and temperature on Removal efficiency of cresol

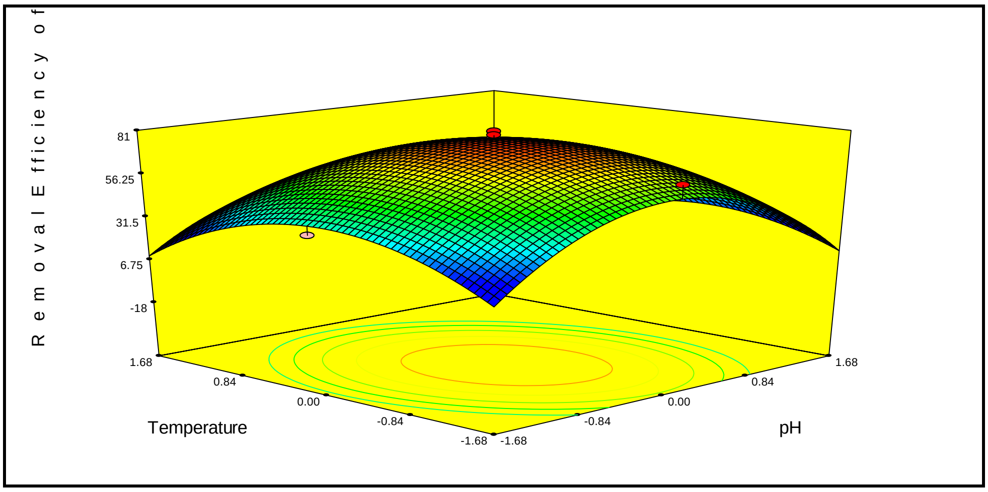

Fig. 4 3D plot showing the effect of temperature and pH on Removal efficiency of cresol

Validation of the experimental model is tested by carrying out the batch experiment under optimal operation conditions. Three repeated experiments are performed and the results are compared. The removal of cresol obtained from experiments is very close to the actual response predicted by the regression model, which proved the validity of the model. At these optimized conditions the maximum removal efficiency is found to be 80%.

Conclusion

This was the first report applying statistical experimental designs to optimize cresol degradation by *Aspergillus fumigatus*. Results suggested that statistical optimum strategy was an effective tool for optimization of process parameters on cresol degradation and for advancing degradation efficiency by *Aspergillus fumigatus*. This study indicated the excellent ability of *Aspergillus fumigatus* in degrading high-strength phenol.

References

1. United Nations Environmental Program (UNEP). (2006). *Division of Technology, Industry and Economics Chemicals Branch*.
2. U.S. Environmental Protection Agency (USEPA) (2010). Technology transfer air toxic website. *Cresols.*
3. Agency For Toxic Substances and Disease Registry (ATSDR). (2008). Toxicological profile for cresols. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.
4. Asakawa, T.; Ogino, K. and Yamabe, K. (1985). Adsorption of phenol on surface modified carbon black from its aquatic solution. II. Influence of surface-chemical structure of carbon on adsorption of phenol. *Bulletin of the Chemical Society of Japan*, vol. 58, no. 7, p. 2009-2014.
5. Essam, T.; Amin, M.A.; Tayeb, O.; Mattiasson, B. and Guieysse, B. (2010). Kinetics and metabolic versatility of highly tolerant phenol degrading *Alcaligenes* strain TW1. *Journal of Hazardous Materials*, vol. 173, no. 1-3, p. 783-788.
6. Kar, S.; Swaminathan, T. and Baradarajan, A. (1997). Biodegradation of phenol and cresol isomer mixtures by *Arthrobacter*. *World Journal of Microbiology and Biotechnology*, vol. 13, no. 6, p. 659-663.
7. Zlateva, P.V.; Gerginova, M.G.; Manasiev, J.S.; Atanasov, B.K.; Peneva, N.M.; Dimova, N.D. and Alexieva, M. (2005). Kinetic parameters determination of the Phenolic derivatives assimilation by *Trichosporon cutaneum* R57. *Biotechnology and Biotechnological Equipment*, vol. 19, no. 1, p. 93-97.
8. Santos, V.L. and Linardi, V.R. (2004). Biodegradation of phenol by filamentous fungi isolated from industrial effluents-identification and degradation potential. *Process Biochemistry*, vol. 39, no. 8, p. 1001-1006.
9. Kumar, A.; Kumar, S. and Kumar, S. (2004). Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194. *Biochemical Engineering Journal*, vol. 22, no. 2, p. 151-159.
10. El-Naas, M.H.; Al-Muhtaseb, S.A. and Makhlouf, S. (2008). Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *Journal of Hazardous Materials*, vol. 164, no. 2-3, p. 720-725.
11. Massalha, N.; Shaviv, A. and Sabbah, I. (2010). Modeling the effect of immobilization of microorganisms on the rate of biodegradation of phenol under inhibitory conditions. *Water Research*, vol. 44, no. 18, p. 5252-5259.
12. Bergauer, P.; Fonteyne, P.A.; Nolard, N.; Schinner, F. and Margesin, R. (2005). Biodegradation of phenol and phenol-related compounds by psychrophilic and cold-tolerant alpine yeasts. *Chemosphere*, vol. 59, no. 7, p. 909-918.
13. Seo, J.S.; Keum, Y.S. and Li, Q.X. (2009). Bacterial degradation of aromatic compounds. *International Journal of Environmental Research and Public Health*, vol. 6, no. 1, p. 278-309.
14. Vasu, A.E. (2008). Removal of phenol and *o*-cresol by adsorption onto activated carbon. *E-Journal of Chemistry,* vol. 5, no. 2, p. 224-232.
15. Pazarlioglu, N.K. and Telefoncu, A. (2005). Biodegradation of phenol by *Pseudomonas putida* immobilized on activated pumice particles. *Process Biochemistry*, vol. 40, no. 5, p. 1807-1814.

*****