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Optimization of Process Variables for the Batch Degradation of Phenol by *Pseudomonas fluorescence* Using Response Surface Methodology

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Abstract: In this study, the classical method of one-variable at a time bioprocess design and Response Surface Methodology (RSM) was performed to evaluate the effects of aeration, agitation and temperature on phenol degradation by *Pseudomonas fluorescence*. Experiments were performed as a function of temperature (25-45°C), aeration (1.0-3.5 vvm) and agitation (200-600 rpm). The results of the one-variable at a time bioprocess design showed that percent phenol degradation increased with increased aeration, agitation and temperature up to a value of 3.0 vvm, 300 rpm and 30°C, respectively. Above these respective values, the percent phenol degradation decreased. Furthermore, phenol biodegradation was optimized by 2^3 full-factorial central composite design. Statistical analysis of results revealed that the linear and quadratic terms of these variables had significant effects and evident interactions existing between the temperature and agitation were found to contribute to the response at a significant level. More also, full-factorial central composite design used for the analysis of treatment combinations gave a second-order polynomial regression model, which was in good agreement with experimental results, with $R^2 = 0.9647$ ($p<0.05$). By response surface methodology and multistage Monte- Carlo optimization technique, the optimal degradation (fermentation) parameters for enhanced phenol degradation were obtained. The optimum process conditions for maximizing phenol degradation (removal) were recognized as follows: temperature 30°C, aeration 3.0 vvm and agitation 300 rpm and the model predicted a maximum percent phenol degradation of 60.7% at these optimum process conditions. This confirmed the closeness of the model to the experimental result of 60.8%.

Key words: *Pseudomonas fluorescence*, phenol, biodegradation, regression model, statistical optimization

INTRODUCTION

Phenol and its derivatives is the basic structural unit in a wide variety of synthetic organic compounds (Annadurai *et al*., 2000). It is an organic, aromatic compound that occurs

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naturally in the environment (Prpich and Daugulis, 2005), but is more commonly produced artificially from industrial activities such as petroleum processing, plastic manufacturing, resin production, pesticide production, steel manufacturing and the production of paints and varnish (Mahadevaswamy *et al*., 1997; Bandyopadhyay *et al*., 1998). This aromatic compound is water-soluble and highly mobile (Collins and Daugulis, 1997) and as such waste waters generated from these industrial activities contain high concentrations of phenolic compounds (Chang *et al*., 1998) which eventually may reach down to streams, rivers, lakes and soil, which represent a serious ecological problem due to their widespread use and occurrence throughout the environment (Fava *et al*., 1995).

Phenol is a listed priority pollutant by the US Environmental Protection Agency (1979) and is considered to be a toxic compound by the Agency for Toxic substances and Disease Registry (2003). The adverse effects of phenol on health are well documented (Calabrese and Kenyon, 1991) and death among adults has been reported with ingestion of phenol ranging from 1 to 32 g (Prpich and Daugulis, 2005). The low volatility of phenol and its affinity for water make oral consumption of contaminated water the greatest risk to humans (Prpich and Daugulis, 2005).

A variety of techniques have been used for the removal of phenol from industrial effluents and contaminated waters with bioremediation receiving the most attention due to its environmentally friendly, its, ability to completely mineralize toxic organic compounds and of low-cost (Kobayashi and Rittman, 1982; Prpich and Daugulis, 2005). Microbial degradation of phenol have been actively studied and these studies have shown that phenol can be aerobically degraded by wide variety of fungi and bacteria cultures such as *Candida tropicalis* (Ruiz-Ordaz *et al*., 1998, 2001; Chang *et al*., 1998); *Acinetobacter calcoaceticus* (Paller *et al*., 1995); *Alcaligenes eutrophus* (Hughes *et al*., 1984; Leonard and Lindley, 1998); *Pseudomonas putida* (Hill and Robinson, 1975; Kotturi *et al*., 1991; Nikakhtari and Hill, 2006) and *Burkholderia cepacia* G4 (Folsom *et al*., 1990; Solomon *et al*., 1994).

It has been demonstrated that treatment of small volumes of toxic compounds at the point of emission using specific microbial strains and better bioreactors allows a higher control over the process and higher removal efficiencies (Schroeder *et al*., 1997). The high dependence of enzymatic activity and cellular maintenance requirements on temperature makes it an important quantity. Temperature exerts an important regulatory influence on the rate of metabolism (Ghosh and Swaminathan, 2003). The effects of oxygen supply vary from species to species (Onken and Liefke, 1989). In addition, oxygen mass transfer is aided by agitation rates that create turbulence and shear forces in the cultivation system causing significant influences on the growth rate and product formation (Hoq *et al*., 1995).

In a mechanically agitated bioreactor, agitation increases the rate of mass and heat transfer operations and provides the required degree of mixing of the reactor contents. Insufficient agitation may lead to limitations in the transfer operations and the appearance of regions of insufficient nutrient content or inadequate temperature or pH (Gonzalez *et al*., 2003). As a result, the overall productivity of the process will decline (Namdev *et al*., 1994). Regarding process variables, aeration and agitation rates are the most pertinent. The most adequate aeration must be carefully determined so, to provide oxygen as demanded by the cell population, but avoiding excessive flow rates that can lead to impellers flooding and cause unnecessarily high operating costs. On the other hand, an intense agitation must be provided, but too high agitation rates should be avoided to prevent attrition and metabolic stress in the bacterial population (Toma *et al*., 1991; Enfors *et al*., 2001; Gonzalez *et al*., 2003). So, the problem of optimization of the agitation and aeration conditions is complex and

should be addressed using a statistically based experimental design. Thus, optimization of process variables is recognized to be an essential aspect of successful fermentation (Kumar, 1995).

This study examined the effect of temperature, aeration and agitation on the degradation of phenol by local strains of *Pseudomonas fluorescence* using both the classical method of optimization that involves varying the level of one parameter at a time over a certain range while, holding the rest of the variables constant and statistical optimization technique for multivariable effect.

MATERIALS AND METHODS

Materials

The microorganism, *Pseudomonas fluorescence* being an indigenous bacteria strain isolated from an oil-polluted area in Niger-Delta region of Nigeria was procured from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The study was conducted in 2008. The microorganism was maintained on nutrient agar slant and stored at 4±1°C for further use.

Methods

Culture Medium and Inoculum

The mineral salt medium used was modified from the one suggested by Bettmann and Rehm (1984). The medium had the following composition per litre: 700 mL deionized water, 100 mL buffer solution A, 100 mL trace elements solution B, 50 mL solution C and 50 mL solution D. Compositions of each solution were as follows: Buffer solution A composition K_2HPO_4 1.0 g, KH_2PO_4 0.5 g, $(NH_4)_2SO_4$ 0.5 g, deionized water 100 mL. Trace element solution B composition NaCl 0.5 g, $CaCl_2$ 0.02 g, $MnSO_4$ 0.02 g, $CuSO_4.5H_2O$ 0.02 g, H_3BO_3 0.01 g, deionized water 50 mL. Solution C composition $MgSO_4.7H_2O$ 0.5 g, deionized water 50 mL, solution D composition $FeSO_4$ 0.02 g, Molybdenum powder 0.02 g, deionized water 50 mL. To prevent the precipitation of $CaSO_4$ and $MgSO_4$ in storage, the water, buffer solution A, trace elements solution B, solution C and solution D were autoclaved at 121°C for 15 min. After cooling, all the solutions were then mixed together and kept as stock solution from which known quantities were taken for the cultivation of the microorganisms.

A primary culture was prepared by transferring two loops full of microorganisms from an agar slant culture into 100 mL of feed medium containing 20 mL of mineral salt medium and 80 mL of 50 mg phenol solution in a 250 mL Erlenmeyer conical flask. This was then, incubated in a New Brunswick gyratory shaker (G25-R model, N.J., USA) for 48 h at a temperature of 30°C and agitated with a speed of 120 rpm. Thereafter, 10 mL of the primary culture was transferred into another 100 mL of feed medium in a 250 mL Erlenmeyer conical flask and the incubation process was repeated. This was the secondary culture that was used as the inoculum for the degradation studies as this ensures that the organisms had fully adapted to growth on the phenol as sole source of carbon and energy.

Optimization Studies

To optimize the range of experimentation for the 2^3 full-factorial central composite design, the following experiments were carried out in a New Brunswick Microferm Twin Bioreactor (PH-22 model, N.J., USA) with 4 L working volume. Eight hundred milliliter of the autoclaved mineral salt medium and 3 L of phenol solution (100 mg L^{-1}) were measured into the bioreactor vessel and 200 mL of the inoculum was introduced aseptically to make up 4 L of working volume. The bioreactor was operated for 48 h at different temperature (25, 30,

Table 1: Experimental range and the levels of the variables

Dependent variable	-α	-1	0	+1	+α
Temperature (X_1) (°C)	21.60	25.0	30.0	35.0	38.40
Aeration (X_2) (vvm)	2.16	2.5	3.0	3.5	3.84
Agitation (X_3) (rpm)	132.00	200.0	300.0	400.0	568.00

Table 2: Coded and uncoded full-factorial central composite design for the three independent variables

	Temperature		Aeration		Agitation	
Experiment No.	Code	Value (°C)	Code	Value (vvm)	Code	Value (rpm)
1	-1	25.0	-1	2.50	-1	200
2	+1	35.0	-1	2.50	-1	200
3	-1	25.0	+1	3.50	-1	200
4	+1	35.0	+1	3.50	-1	200
5	-1	25.0	-1	2.50	+1	400
6	+1	35.0	-1	2.50	+1	400
7	-1	25.0	+1	3.50	+1	400
8	+1	35.0	+1	3.50	+1	400
9	-1.682	21.6	0	3.00	0	300
10	+1.682	38.4	0	3.00	0	300
11	0	30.0	-1.682	2.16	0	300
12	0	30.0	+1.682	3.84	0	300
13	0	30.0	0	3.00	-1.682	132
14	0	30.0	0	3.00	+1.682	568
15	0	30.0	0	3.00	0	300
16	0	30.0	0	3.00	0	300
17	0	30.0	0	3.00	0	300
18	0	30.0	0	3.00	0	300
19	0	30.0	0	3.00	0	300
20	0	30.0	0	3.00	0	300

35, 40 and 45°C); aeration (1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 vvm) and agitation rates (200, 300, 400, 500 and 600 rpm). Culture broth was withdrawn at every 6 h for phenol determination. From the above experiments, the range of temperature, aeration and agitation rates were chosen for further optimization of the design.

Design of Experiments

Temperature (25, 30 and 35°C); aeration (2.5, 3.0 and 3.5 vvm) and agitation (200, 300 and 400 rpm) that served as critical variables X_1, X_2 and X_3 respectively are the range and levels of the process variables under study (Table 1). The actual design of experiments is given in Table 2 for each experiment that was carried out in a New Brunswick Microferm Twin Bioreactor containing 800 mL mineral salt medium, 3 L of phenol solution (100 mg L^{-1}) and 200 mL of inoculum that was operated for 48 h. Samples were withdrawn after 48 h for phenol determination.

Phenol Determination

The undegraded phenol was estimated quantitatively by the spectrophotometric method using 4-amino antipyrene as color indicator (Yang and Humphrey, 1975) at an absorbance of 510 nm.

Response Surface Methodology and Statistical Optimization

A full-factorial central composite design using response surface methodology (Khuri and Cornell, 1987; Montgomery, 1991; Ghosh and Swaminathan, 2003; Preetha *et al*., 2007; Cao *et al*., 2008) is a powerful tool for understanding complex processes. The full-factorial central composite design consists of (a) a complete 2^k factorial design where,

k (= 3) is the number of tests variables, (b) n_o center points (n_o>1) and (c) two axial points on the axis of each design variables at a distance of α ($\alpha = 2^k/4 = 1.682$ for k = 3) from the design center (Khuri and Cornell, 1987; Ghosh and Swaminathan, 2003). Hence, the total number of design points is $N = 2^k + 2k + n_o$ and these data are fitted in a second order polynomial model (Ghosh and Swaminathan, 2003). An orthogonal 2^3 full-factorial central composite design with 6 replicates (n_o = 6) at the central point, all in duplicates resulting in total of 20 experiments were used to optimize the chosen key variables that have effect on phenol microbial degradation are given in Table 1. The variables were coded according to the Eq. 1 (Box and Behnken, 1960; Box and Draper, 1959; Annadurai *et al*., 2000).

$$x_i = (X_i - X_o)/\Delta X_i \quad (1)$$

where, x_i, X_i and X_o are the coded value, uncoded value and the value at the center point respectively of the ith test variable and Δx_i is the step change value. The full experimental design in coded and uncoded form is given in Table 2. The behavior of the system was explained by the following second order polynomial equation:

$$Y = \beta_o + \Sigma\beta_i X_i + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_{ij} \quad (2)$$

where, Y is predicted response, β_o is offset term, β_i is linear effects, β_{ii} is quadratic effects, β_{ij} is interaction effects. X_i (i = 1, 2, 3) and X_j (j = 1, 2, 3) ($i \neq j$) are the process variables.

The graphical representation of these equations are called response surfaces which was used to describe the individual and cumulative effects of the test process variables on the response and to determine the mutual interactions between the test variables and their subsequent effect on the response (Ghosh and Swaminathan, 2003).

A statistical program package, Design Expert (Start-Ease Inc, Minneapolis, MN) was used for regression analysis of the data obtained and to estimate the coefficient of the polynomial equation.

RESULTS AND DISCUSSION

Classical Method of Optimization: Effect of Aeration, Agitation and Temperature

The effects of aeration rate, agitation rate and temperature on phenol degradation by indigenous (local strains) *Pseudomonas fluorescence* were studied. It could be seen that the rate of degradation (percent degradation) increased with increase in aeration up to an aeration rate of 3.0 vvm, above which it decreased (Fig. 1). It was observed that the biomass concentration increased up to an aeration rate of 3.0 vvm and above that, it decreased. The increase in degradation rate may be due to the fact that as aeration rate increased more dissolved oxygen (i.e., higher mass transfer) were made available for the metabolism of the organism, while the decrease may be due to toxic effect of the excess air (oxygen). Oboirien *et al.* (2005) and Hannaford and Kuek (1999) also gave a similar report that biodegradation rate increased with increase in aeration rate when they considered an aeration rate of 1.5 to 2.5 vvm and 4 to 16 vvm, respectively. Oboirien and his co-workers used freely suspended *Pseudomonas fluorescence* NCIB 3756 and a New Brunswick Microferm Twin Fermentor for their studies, while; Hannaford and Kuek (1999) used an immobilized *Pseudomonas putida* ATCC 11172 and a bubble column reactor for their studies. Collins and Daugulis (1997) reported that aeration rate greater than 0.5 vvm produce excessive foaming and led to solvent and cell losses. In this work, no foaming was observed and this is in agreement with the observation of Oboirien *et al*. (2005).

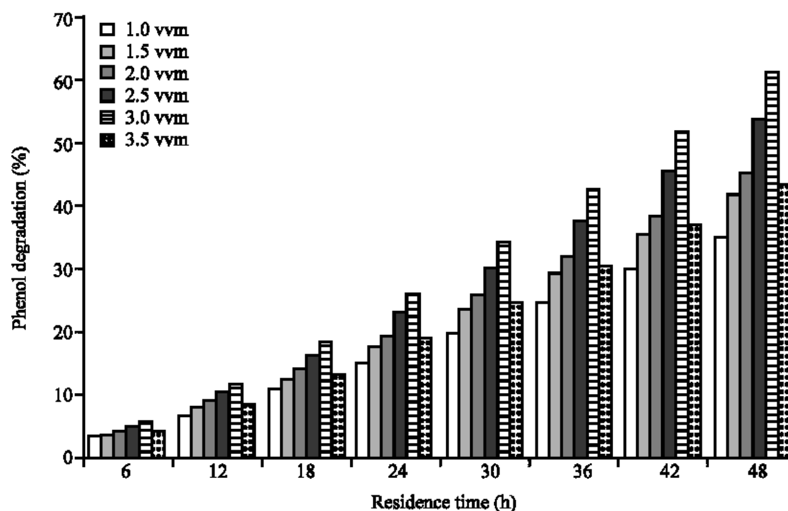

Fig. 1: Effect of aeration rate on phenol degradation by *P. fluorescence*

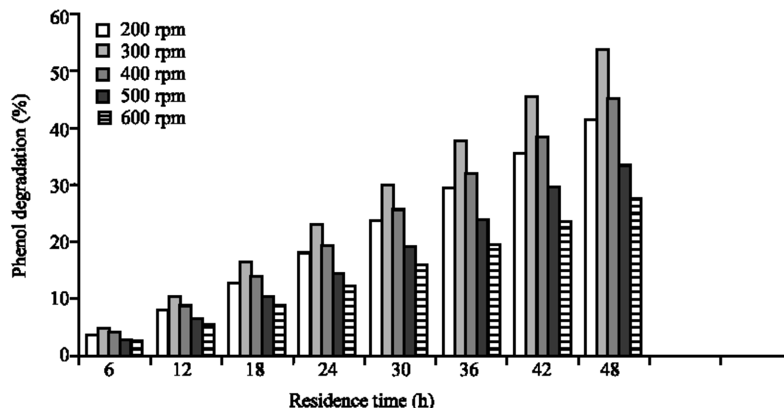

Fig. 2: Effect of agitation rate on phenol degradation by *P. fluorescence*

It may be seen that the rate of phenol degradation increased from an agitation rate of 200 to 400 rpm and above 400 rpm the rate decreased (Fig. 2). It was observed that the biomass concentration increased up to an agitation rate of 400 rpm and above that, it decreased. Sobczuk *et al*. (2006) reported a similar observation. The increase in biodegradation rate may be due to adequate high mass transfer thus allowing more oxygen to be dissolved and made available for the metabolism of the organism. While, the decrease may be due to higher shear stress effect thus leading to cell loss or lower biomass concentration (Hoq *et al*., 1995). However, the optimum agitation rate was found to be 300 rpm at which the percent degradation was highest.

Also, it could be seen that the phenol degradation rate (percent degradation) increased with increase in temperature up to 40°C above which, it decreased (Fig. 3). In this study, it was observed that the biomass concentration increased up to a temperature of 40°C and above that, it decreased. The decrease in the rate of degradation may be due to decrease in the effective reactivity of the multi-enzyme complex system within the cell. A similar

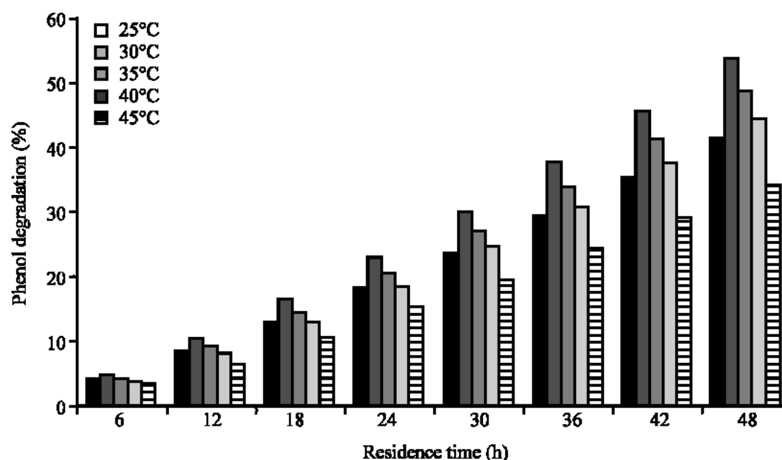

Fig. 3: Effect of temperature on phenol degradation by *P. fluorescence*

Table 3: Experimental and theoretically predicted values for degradation of phenol by *Pseudomonas fluorescence*

Run	Experimental value (%)	Predicted value (%)
1	34.2	38.1
2	37.8	41.3
3	30.6	34.3
4	34.9	37.9
5	40.8	41.9
6	43.2	43.6
7	38.5	39.1
8	41.1	41.2
9	49.2	45.7
10	52.4	50.2
11	47.5	44.4
12	41.7	39.2
13	36.6	30.1
14	35.3	36.0
15	60.4	60.7
16	60.7	60.7
17	60.8	60.7
18	60.8	60.7
19	60.6	60.7
20	60.5	60.7

observation was reported by Bandyopadhyay *et al.* (1998) that biodegradation rate of phenol by *Pseudomonas putida* MTCC 1194 increased with increase in temperature from 15 to 30°C above which it decreased. However, from the same Fig. 3, it may be observed that the highest percent degradation was at a temperature of 30°C and thus represent the optimum temperature.

Optimization of Temperature, Aeration and Agitation Using Response Surface Methodology

The quantitative description of the process variables (physical condition) effects on phenol microbial degradation was performed. The optimum level of aeration, agitation and temperature are as shown in Fig. 1-3. Aeration (2.5, 3.0, 3.5) vvm, agitation (200, 300, 400) rpm and temperature (25, 30, 35)°C, were optimized using the 2^3 full factorial central composite design of experiments. Response surface methodology is an empirical modeling technique involved in the evaluation of the relationship of a set of controlled experimental factors and

Table 4: Regression Analysis for the degradation of phenol by *P. fluorescence*, quadratic response surface model fitting (ANOVA)

Source	Sum of squares	df	Mean square	F-value	p-value
Model	21.02	9	2.336	30.39	0.0001
Residual (error)	0.77	10	0.077		
Correlation total	21.79	19			
$R^2 = 0.9647$ Adj $R^2 = 0.9329$					

Table 5: Coefficient of the model for *P. fluorescence*

Variables	Coefficient value	Standard error	t-value	p-value
β_0	60.74	1.13	53.62	-
β_1	1.33	0.75	1.77	0.112
β_2	-1.54	0.75	-2.05	0.040
β_3	1.76	0.75	2.35	0.060
β_{11}	-4.53	0.57	-7.95	0.000
β_{22}	-6.72	0.57	-11.80	0.000
β_{33}	-9.80	0.57	-17.20	0.000
β_{12}	0.11	0.98	0.11	0.602
β_{13}	-0.37	0.98	-0.38	0.720
β_{23}	0.26	0.98	0.27	0.540

observed results (Annadurai *et al*., 2000). The average percent phenol degraded at each fermentation run is summarized and presented in Table 3 along with predicted values. The experimental data were fitted to a second order polynomial regression model containing 3 linear, 3 quadratic and 3 interaction terms using the same experimental design software. The regression equation obtained after analysis of variance gives the level of degradation of phenol as a function of the different process variables: aeration, agitation and temperature. All terms regardless of their significance are included in the following Eq. 3:

$$Y = 60.74 + 1.33X_1 - 1.54X_2 + 1.76X_3 - 4.53X_1^2 - 6.72X_2^2 - 9.80X_3^2 + 0.11X_1X_2 - 0.37X_1X_3 + 0.26X_2X_3 \quad (3)$$

where, X_1, X_2 and X_3 represent coded values of temperature, aeration and agitation respectively and Y is the response variable (maximum degradation of phenol).

The optimum coded and uncoded value was obtained by solving equation 3 analytically. The optimum coded and uncoded value of temperature was found to be 0.01 and 30°C, respectively. While for aeration, it was obtained as -0.01 and 3.0 vvm, respectively and for agitation, it was found to be 0.01 and 300 rpm, respectively at maximum percent age of phenol degradation 60.7%. The optimum temperature for the biodegradation of phenol has generally been reported to be 30°C (Bandyopadhyay *et al*., 1998; Polymenakou and Stephanou, 2005).

The Analysis of Variance (ANOVA) of the regression model demonstrates that the model is highly significant (Table 4), as is evident from Fisher F_{test} ($F_{model} = 30.39$) and a very low failure probability ($p = 0.0001$). Moreover, the computed F value ($F_{0.01(9, 10)} = 30.39$) was greater than the tabular F value ($F = 4.94$) at the 1% level indicating that the treatment differences are highly significant. The value of R (= 0.9822) indicates a high degree of correlation between the observed value and predicted values. The value of the determination coefficient ($R^2 = 0.9647$) being a measure of goodness of fit to the model indicates that only about 4% of the total variations are not explained by the model. The Coefficient of Variation (CV) indicates the degree of precision with which the treatment is compared. Usually the higher the value of CV, the lower is the reliability of the experiment (Ghosh and Swaminathan, 2003). Here, the low value of CV (= 5.98%) indicates a greater reliability of the experiment performed.

The coefficient of the model (parameter estimation) and the corresponding p-values (Table 5) suggest that among the test variables, linear and quadratic effect of aeration and

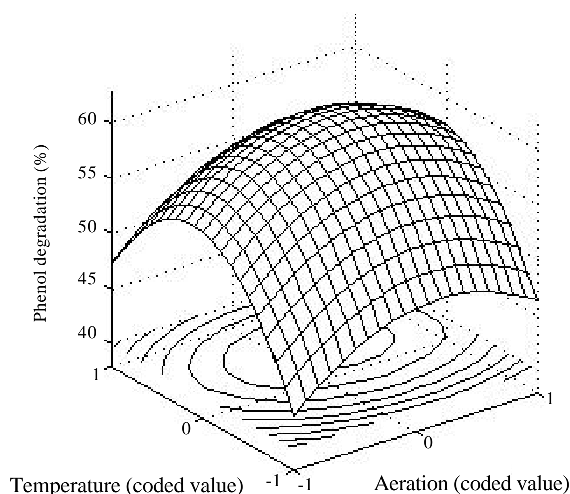

Fig. 4: Phenol degradation by *Pseudomonas fluorescence* on 3-D graphics for response surface optimization vs. temperature and aeration

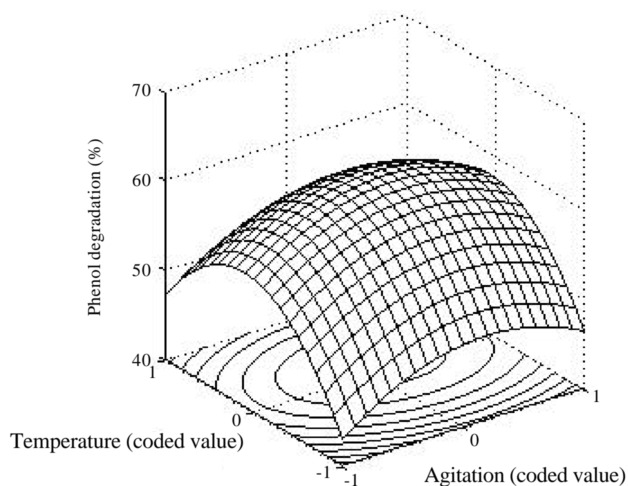

Fig. 5: Phenol degradation by *Pseudomonas fluorescence* on 3-D graphics for response surface optimization vs. temperature and agitation

agitation are highly significant. The quadratic effect of temperature was more pronounced than linear effect. These observations can be interpreted as a consequence of proportional relationship between the variables and phenol degradation. The mutual effects of temperature and agitation and, aeration and agitation are approximately of equal significance than the other interaction. This data analysis also substantiates the inference that can be drawn from three-dimensional contour plots (3-D graphics) as shown in Fig. 4-6, respectively. The interactions amongst temperature, aeration and agitation are quite prominent from the elliptical nature of the respective contour plots. These figures also suggest the optimum range of the process variables.

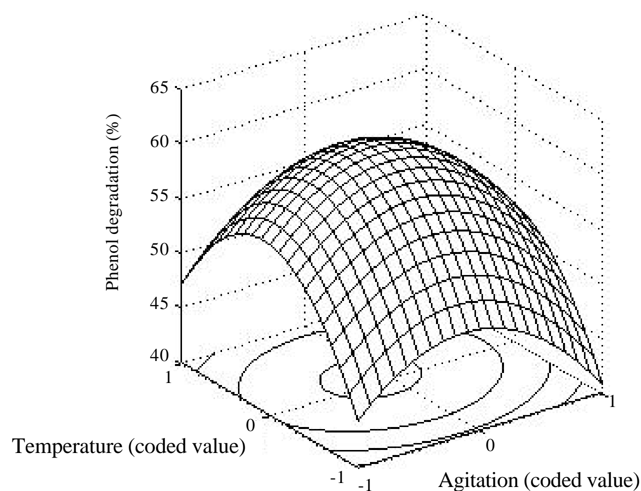

Fig. 6: Phenol degradation by *Pseudomonas fluorescence* on 3-D graphics for response surface vs. temperature and agitation

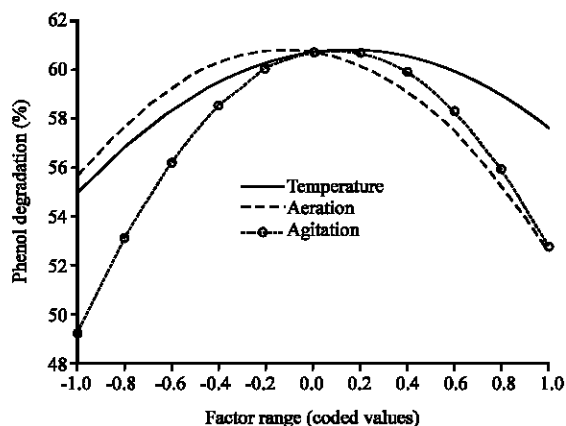

Fig. 7: Factor plot representing the individual variables effect on phenol degradation of *Pseudomonas fluorescence*

Factor Plot

The factor effect function plot (Fig. 7) was used to assess the effect of each factor graphically. From the trace plot as shown in Fig. 7, it can be seen that each of the three variables used in the present study has its individual effect on phenol biodegradation by *P. fluorescence*. Gradual increase in temperature, aeration and agitation rates from low level (coded value-1) to a higher level (coded value +1) resulted in both increase and decrease of phenol degradation. Moreover, it is also to be noted from Fig. 7 that over the range of agitation (200 to 400 rpm) the phenol degradation changed in a wide range, which was not the case for temperature and aeration. This clearly indicates that keeping temperature and aeration at the optimum level a change in agitation affects the process more severely than done otherwise. The optimum values were found by solving the regression equation

analytically. The highest phenol degradation that can be achieved according to the model prediction under the optimal experimental conditions is 60.7%. The experimental results indicated, a degradation of about 60.8% under the optimal process conditions. This confirms the closeness of the model to the experimental results.

CONCLUSION

The present study showed the potential of the isolated indigenous *P. fluorescence* for phenol wastewater treatment. The performance of the indigenous strain in biodegradation of phenol in the nutrient medium is excellent. The response surface methodology using 2^3 full-factorial composite designs was adopted to optimize the process variables like temperature, aeration and agitation for the microbial degradation of phenol using a mechanically agitated bioreactor. The design generated may be used for designing a treatment plant for phenol waste effluents where, collection can be achieved on a large scale. The statistical analyses and the closeness of the experimental results and model predictions show the reliability of the regression model.

REFERENCES

Agency for Toxic Substances and Disease Registry, 2003. Medical Management Guidelines for Phenol. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Annadurai, G., S.M. Balan and T. Murugesan, 2000. Design of experiments in the biodegradation of phenol using immobilized *Pseudomonas pictorium* (NICM-2077) on activated carbon. Bioproc. Eng., 22: 101-107.

Bandyopadhyay, K., D. Das and B.R. Maiti, 1998. Kinetics of phenol degradation using *Pseudomonas putida* MTCC 1194. Bioproc. Eng., 18: 373-377.

Bettmann, H. and H.J. Rehm, 1984. Degradation of phenol by polymer entrapped microorganisms. Applied Microbial. Biotechnol., 20: 285-290.

Box, G.E.P. and D.W. Behnken, 1960. Some new three level designs for the study of quantitative variables. Technometrics, 2: 455-475.

Box, G.E.P. and N.R. Draper, 1959. A basis for the selection of a response surface design. J. Am. Stat. Soc., 54: 622-654.

Calabrese, E.J. and E.M. Kenyon, 1991. Air Toxics and Risk Assessment. Lewis Publishers, Chelsea, MI., pp: 98.

Cao, Y., M. De Jing, J. Lu and J. Long, 2008. Statistical optimization of xylanase production by *Aspergillus niger* AN-13 under submerged fermentation using response surface methodology. Afr. J. Biotechnol., 7: 631-638.

Chang, Y.H., C.T. Li, M.C. Chang and W.K. Shieh, 1998. Batch phenol degradation by *Candida tropicalis* and its Fusant. Biotechnol. Bioengin., 60: 391-395.

Collins, L.D. and A.J. Daugulis, 1997. Biodegradation of phenol at high initial concentration in two-phase partitioning batch and fed-batch bioreactors. Biotechnol. Bioeng., 55: 155-162.

Enfors, S.O., M. Jahhic, A. Rozkov, B. Xu and M. Hecker *et al.*, 2001. Physiological responses to mixing in large scale bioreactors. J. Biotechnol., 85: 175-185.

Fava, F., P.M. Armenante, D. Kafkewitz and L. Marchetti, 1995. Influence of organic and inorganic growth supplements on the aerobic biodegradation of chlorobenzoic acid. Applied. Microbiol. Biotechnol., 43: 171-177.

Folsom, B.R., P.J. Champan and P.H. Pritchard, 1990. Phenol and Trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates. Applied Environ. Microbial., 56: 1279-1285.

Ghosh, S. and T. Swaminathan, 2003. Optimization of process variables for the extractive fermentation of 2, 3 butanediol by *Klebsiella oxytoca* in aqueous two-phase system using response surface methodology. Chem. Biochem. Eng. Q., 17: 319-325.

Gonzalez, R., J.C. Gentina and F. Acevedo, 2003. Optimization of the solids suspension conditions in a continuous stirred tank reactor for the biooxidation of refractory gold concentrates. Electronic J. Biotechnol., 6: 1-8.

Hannaford, A.M. and C. Kuek, 1999. Aerobic batch degradation of phenol usingiImmobilized *Pseudomonas putida*. J. Indust. Microbiol. Biotechnol., 22: 121-126.

Hill, G.A. and C.W. Robinson, 1975. Substrate inhibition kinetics: Phenol Degradation by *Pseudomonas putida*. Biotechnol. Bioeng., 17: 599-615.

Hoq, M.M., B.O. Solomon, C. Hempel, U. Rinas and W.D. Deckwer, 1995. The kinetics of cellulase-free xylanase excretion by *Thermomyces lanugiosus* RT 9. J. Chem. Technol. Biotechnol., 63: 229-236.

Hughes, E.J., R.C. Bayly and R.A. Skurray, 1984. Evidence for isofunctional enzymes in the degradation of phenol, m-and p-toluate and p-cresol via Catechol meta cleavage pathways in *Alcaligenes eutrophus*. J. Bacteriol., 158: 79-83.

Khuri, A.L. and J.A. Cornell, 1987. Response Surfaces: Design and Analysis. Marcel Dekker, New York.

Kobayashi, H. and B.E. Rittman, 1982. Microbial removal of hazardous organic compounds. Environ. Sci. Technol., 16: 170-183.

Kotturi, G., C.W. Robinson and W.E. Inniss, 1991. Phenol degradation by a Psychtrotrophic strain of *Pseudomonas putida*. Applied Microbial. Biotechnol., 34: 539-543.

Kumar, A.S., 1995. Studies on microbial production of 2,3 butanediol by K. oxytoca in batch, continuous and cell recycle systems. Ph.D. Thesis, IIT. Madras, India.

Leonard, D. and N.D. Lindley, 1998. Carbon and energy flux constraints in continuous cultures of *Alcaligenes eutrophus* grown on phenol. Microbiology, 144: 241-248.

Mahadevaswamy, M., I.D. Mall, B. Prasad and I.M. Mishra, 1997. Removal of phenol by adsorption on coal fly ash and activated carbon. Poll. Res., 16: 170-175.

Montgomery, D.C., 1991. Design and Analysis of Experiments. 3rd Edn., John Wiley and Sons Inc., USA., ISBN: 1566765617.

Namdev, P.K., E.H. Dunlop, K. Wenger and P. Villeneuve, 1994. Role of turbulence in fermentations. In: Advances in Bioprocess Engineering, Galindo, E. and O.T. Ramírez (Eds.). Kluwer Academic Publishers, Dordrecht, pp: 149-156.

Nikakhtari, H. and G.A. Hill, 2006. Continuous bioremediation of phenol-polluted air in an external loop airlift bioreactor with a packed bed. J. Chem. Technol. Biotechnol., 81: 1029-1038.

Oboirien, B.O., B. Amigun, T.V. Ojumu, O.A. Ogunkunle, O.A. Adetunji, E. Betiku and B.O. Solomon, 2005. Substrate inhibition kinetics of phenol degradation by *Pseudomonas aeruginosa* and *Pseudomonas fluorescence*. Biotechnology, 4: 56-61.

Onken, U. and E. Liefke, 1989. Effect of Total and Partial Pressure (Oxygen and Carbon Dioxide) on Aerobic Microbial Processes. In: Advances in Biochemical Engineering Biotechnology, Fiechter, A. (Ed.). Vol. 40. Springer Verlag, Berlin, pp: 137-169.

Paller, G., R.K. Hommel and H.P. Kleber, 1995. Phenol degradation by *Acinetobacter calcoaceticus* NCIB 8250. J. Basic Microbiol., 35: 325-335.

Polymenakou, P.N. and E.G. Stephanou, 2005. Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil *Pseudomonas*. Biodegradation, 16: 403-413.

Preetha, R., N.S. Jayaprakash, R. Philip and B.I.S. Singh, 2007. Optimization of medium for the production of a novel aquaculture probiotic *Micrococcus* MCCB 104 using central composite design. Biotechnol. Bioproc. Eng., 12: 548-555.

Prpich, G.P. and A.J. Daugulis, 2005. Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two-phase partitioning bioreactor. Biodegradation, 16: 329-339.

Ruiz-Ordaz, N., J.C.R. Lagunez, J.H.C. Gonzalez, E.H. Manzano, E.C. Urbina and J.G. Mayer, 1998. Growth kinetic model that describes the inhibitory and lytic effects of phenol on Candida tropicalis yeast. Biotechnol. Prog., 14: 966-969.

Ruiz-Ordaz, N., J.C. Ruiz-Lagunez, J.H. Castanon-Gonzalez, E. Hernandez-Manzano, E. Cristiani-Urbina and J. Galindez-Mayer, 2001. Phenol biodegradation using a repeated batch culture of Candida tropicalis in a multistage bubble column. Revista Latinoamericana de Microbiologia, 43: 19-25.

Schroeder, M., C. Muller, C. Posten, W.D. Deckwer and V. Hecht, 1997. Inhibition kinetics of phenol degradation from unstable steady state data. Biotechnol. Bioeng., 54: 567-576.

Sobczuk, T.M., F.G. Camacho, E.M. Grima and Y. Christi, 2006. Effects of agitation on the micoalgae *Phaeodactylum tricomutum* and *Porphyridium cruentum*. Bioproc. Biosyst. Eng., 28: 243-250.

Solomon, B.O., C. Posten, M.P.F. Harder, V. Hecht and W.D. Deckwer, 1994. Energetics of *Pseudomonas cepacia* growth in a chemostat with phenol Limitation. J. Chem. Technol. Biotechnol., 60: 275-282.

Toma, M.K., M.P. Ruklisha, J.J. Vanags, M.O. Zeltina and M.P. Leite *et al*., 1991. Inhibition of microbial growth and metabolism by excess turbulence. Biotechnol. Bioeng., 38: 552-556.

US Environmental Protection Agency, 1979. Phenol Ambient Water Quality Criteria. Office of Planning and Standards, EPA., Washington, DC., pp: 296-786.

Yang, R.D. and A.E. Humphrey, 1975. Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. Biotechnol. Bioeng., 17: 1211-1235.