Isolation And Identification Of Phenol Degrading Microorganism- Optimization Of Process Parameters Using Box- Behnken Design

SRIDEVI V *[1], RANJITHA M[2], DAYANA K[3] , SATYA[4] AND DIVYATEJA D[5]

[1] Professor, Department of Chemical Engineering, Andhra University, Visakhapatnam.
[2]M.Tech, Department of Chemical Engineering, Andhra University, Visakhapatnam.
[3]Associate Professor, Visakha Institute of Engineering & Technology, Visakhapatnam.
[4] Women Scientist, Center for Biotechnology, Department of Chemical Engineering, Andhra University, Visakhapatnam.
[5]PhD Scholar, Department of Chemical Engineering, Andhra University, Visakhapatnam.

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Abstract- The present study indicated the ability of isolated R3 strain in degrading high-strength phenol. Optimal conditions obtained in this experiment laid a solid foundation for further use of this microorganism in the treatment of phenol effluents. The growth and phenol biodegradation study was carried out in MSAM medium with phenol as the sole carbon source and energy. The strains were designated as R1, R2, R3, R4, R5 and R6. One of the strains namely R3 was found to be highly effective for the removal of phenol. The effect of contact time, initial phenol concentration, temperature and pH and on the rate of phenol degradation by that particular strain was carried out. Observations revealed that the optimal conditions for phenol removal were found to be pH of 8, temperature of 36°C, contact time of 96 hrs and initial concentration of phenol of 200 ppm. Box Behnken Design" (BBD) was used to optimize the process of phenol degradation by R3 strain, isolated from textile effluent. In RSM-BBD method, high and low values were assigned for the three variables viz. concentration, contact time and temperature. The results of BBD method showed the significant effect of temperature, contact time and concentration on phenol removal from aqueous solution. The predicted results showed that the maximum removal efficiency of phenol (91%) could be obtained under the optimum conditions of contact time 100 hrs, concentration 221.4 ppm and temperature 35.6°C. These predicted values were further verified by validation experiments. The excellent correlation between predicted and experimental values confirmed the validity and practicability of this statistical optimum strategy.

Index Terms- Biodegradation; Identification; phenol; Optimization; BBD.

I. INTRODUCTION

With the advent of rapid industrialization, Indian economy had progressed; however the industrial boon would also spread their deadly tentacles and engulf the environment, eventually becoming a major cause of air, water and land pollution. The contribution of industries towards pollution is clearly understood in the recent times. The pollutants from industrial discharge and sewage besides finding their way to surface water reservoirs and rivers are also percolating into ground to pollute ground water sources most importantly may contain toxic chemicals that disturb the ecosystem and pose deadly effects on human, animal health, subdue plant growth and toxicants easily enter the food chain. Hence, the past four decades had witnessed a number of treatment technologies to overcome such pollution problems. Pollutants resembling structural features of xenobiotics [1] mostly include organic sulfonic acids, halogenated aliphatic and polycyclic aromatic hydrocarbons, s-triazines, nitroaromatic compounds, azo compounds and synthetic polymers. Polycyclic aromatics, nitroaromatic compounds (NACs), and other hydrocarbons (PAHs) constituting crude oil, are among the diverse group of xenobiotic chemicals responsible for immense environmental pollution.

Environmental preservation has become a key issue in a society because it is often linked to quality of life. Organic compounds are the major water pollutants, among which phenol and its analogous has been the subject of great concern, as they are toxic in nature and induce adverse effect on receiving bodies. It is non-persistent in the environment and the major part of phenol in the atmosphere is degraded by photochemical reactions. A minor part will be removed by rain. Phenol in water and soil is degraded by abiotic reactions and microbial activity. They induce genotoxic, carcinogenic, immunogenic, haematological& physiological effects and have a high bioaccumulation rate along the food chain due to its lipophilicity. The World Health Organization (WHO) recommends the threshold permissible phenolic concentration of $0.001 mgL^{-1}$ in portable waters and threshold concentration of phenol in drinking water below 1.0 mg/L. While Ministry of Environment and Forests (MoEF), Government of India, have set a maximum concentration level of 1.0 mgL^{-1} of phenol in the industrial effluents for safe discharge into surface waters. Thus, the treatment of phenol is necessitated which is done either by conventional or biological techniques [2]. Favourably, biological degradation is generally preferred as it has advantages of lower costs and possibility of complete mineralization [3] therefore does not give rise to any hazardous by-products.

The aim of this study is to investigate the ability of newly isolated bacteria to degrade phenol and to optimize most

significant parameters using Box- Behnken design. In the present investigation, attempts were made to isolate phenol degrading microorganisms from industrial effluents and enrichment to select the most efficient strain for phenol biodegradation.

II. MATERIALS AND METHODS

2.1 Sample Collection: The effluent samples were collected from Textile industry

2.2 Sample Preparation: The samples were collected in sterile bottles Stored it in refrigerator at 4°C. All the samples were used within 7 days from the day of collection for bacteriological analysis [4]. The water sample Bottles were mechanically shaken prior to use and Kept for 10 minutes to allow heavy particles to settle down. The approximate volume of upper layer of water was taken for bacteriological analysis.

2.3 Fourier Transform Infrared Spectroscopy (FTIR) analysis: FT-IR is used to determine the different functional group such as alcohol, alkane, alkynes, alkenes and other such groups present in the substance which here is Phenol [5]. Interpreting infrared (IR) spectra is of immense help to structure determination. Not only will it tell you what functional groups and structural elements are there, it will also clarify which ones are present, and also concentration of bands by using values of transmittance.

2.4 Steam Distillation: Steam distillation is a special type of distillation (a separation process) for temperature sensitive materials like natural aromatic compounds. The steam distillation set up that contains the Boiling Flask (1L), Biomass Flask (2L), and Distillation Arm, Glass Stopper for Distillation Arm, Condenser and Erlenmeyer flask. The distillation set up was shown in the Figure 1.

Figure 1: Steam Distillation Setup

2.5 Isolation and Screening of Phenol degrading microorganisms

The process consists of the following steps

Media Preparation → Sterilization → Culturing Inoculation

2.5.1 Isolation of Microorganisms capable of degradation of phenol

After the cooling takes place, by using laminar flow hood (Product protection from microbial contaminants), an industrial effluent of about 1 ml was suspended in 10 ml of distilled water, stirred well for about 30 min and filtered. From this, 0.1 ml sample was pipette out and surface spread in each Petri plate containing nutrient agar, nutrient agar + 200 mg/l phenol including the petriplate containing mineral salt agar medium (MSAM). All the petriplates were incubated at 37^{0}C for about a week. Regular observations were made.

2.5.2 Screening and selection of phenol degrading microorganisms

After incubation, the representative organisms growing on petriplates were purified. The pure microorganisms were tested for their ability to grow on phenol by inoculating to the MSAM media containing phenol (200ppm). The strains capable of growth at these concentrations were selected. After one week the well defined colonies were purified by streaking on agar plates containing the same medium by streak plate method.

2.6 Maintenance of Phenol resistant isolates

Isolates transferred were grown on MSAM phenol agar slants and sub-cultured. The isolates which were grown well in MSAM were maintained at 37ºC and labelled as R-1, R-2, R-3, R-4, R-5and R-6, were preserved for further studies.

2.7 Study of Growth Kinetics

Strain selection based on phenol acclimatization

The isolated strains R1, R2, R3, R4, R5 and R6 were inoculated into MSM containing phenol as carbon source for 48 hours shaking at 120rpm [6]. After 48 hours, the Cell density was determined spectrophotometrically by measuring turbidity at 600nm.

2.8 Phenol degrading studies

The isolated strain R3 was grown in MSM medium by incubating overnight at 37°c on shaker at 120rpm. The 24 hrs old culture was inoculated into MSM medium with phenol as sole carbon source. Preliminary degrading studies were carried out with addition of isolated strain containing different concentrations of phenol [7], different periods of time, different pH values and different temperature conditions. The reaction mixture containing all components but devoid of isolated culture was used as control. The phenol concentrations were determined by analysing samples at every 24 hrs interval by using UV Spectrophotometer. The residual amount of phenol present in the sample was measured by calorimetric assay 4- amino antipyrine method.

2.8.1 4- Amino Antipyrine method:

When phenol reacts with 4- Amino Antipyrine in the presence of potassium ferricyanide forms coloured antipyrine dye this dye is kept in aqueous solution. Freshly inoculated culture of 5ml was taken by adding 95ml of phenol broth medium containing 1-3g/L phenol on 12 hours interval and then centrifuged at 1200 rpm for 1 hr. Supernatant was collected and sample was prepared for measurement of optical density of phenol. Phenol analysis was carried out by measuring at wavelength 500nm using UV spectrophotometer, after colour development by 4- Amino antipyrine method for the examination of sample.

2.9 Preliminary Studies for determining Optimum conditions:

Selected newly isolated bacterial strains were grown in the nutrient broth by incubation at overnight, at 37^{0}C on shaker at 120rpm. This 24 h young culture used to optimize the following culture conditions of phenol biodegradation with inoculated into

MSM medium which contained 200 mgL^{-1} phenols. Optimization of the parameters like contact time, initial phenol concentration, pH and temperature of phenol degradation by selected newly isolated bacteria was carried out with MSM medium. Phenol degradation experiments were carried out in 250mL shake flask containing 100mL of MSM medium with 200mgL^{-1} of phenol as sole carbon source [8]. The medium was inoculated with R3 strain to initiate the cultivation and degradation of phenol and centrifuged at 1400rpm for 1 hr [9]. The supernatant was withdrawn at regular intervals (24hr) and analyzed for cell growth and phenol concentration using the 4- Amino antipyrine method [10].

Determination of % phenol degradation

The extent to which the test sample was degraded by the microorganism after finding out both the absorbance value in UV-Visible spectrophotometer and its corresponding value of phenol concentration from 4- Amino antipyrine method was calculated using the below given formula.

$$\% \text{ Phenol Degradation} = \frac{\text{Initial concentration-final concentration}}{\text{Initial concentration}} \times 100$$

2.10 Optimization of process parameters using Box- Behnken design:

For optimizing a process with 3 variables at 3 levels, Box-Behnken design of RSM is widely used. Range fixation for process variables is quite crucial when carrying experiments according to any statistical design of experiment, otherwise once the experimentation is over, the optimal conditions obtained by RSM might not be found within the chosen range. On the basis of preliminary studies, the range is fixed for the 3 chosen variables. The parameters selected for optimization by Box-Behnken include temperature(X1), contact time (X2) and concentration (X3). Using the 'MATLAB function regstats', the data obtained was analysed and response surface plots were constructed which indicated the possibility of degradation of phenol. Statistical analysis of the model was performed to evaluate the Analysis of Variance (ANOVA).

III. RESULTS AND DISCUSSION

3.1 Fourier Transform Infrared Spectroscopy (FTIR) analysis:

FTIR analysis was carried out using Bruker FTIR (accessory-ALPHA; software: opus.6.4) at a resolution of 6 cm^{-1} and the changes in % transmission at different wavelengths were observed. In the FTIR analysis, the bands located within the range were due to O–H stretch, H–bonded respectively. The spectra obtained after detection were in the region 3200-3500 cm^{-1} which indicates the presence of phenols (Figure-2).

Figure 2: FTIR Spectrum of the sample

3.2 Steam distillation:

The steam distillate of 100ml was collected and the collected distillate was analysed for phenols by using 4- amino antipyrene method. The concentration of phenol obtained was 46 ppm.

3.3 Isolation and characterization of bacterial strains:

3.3.1 Selective Isolation of Phenol degrading Bacteria:

There are 6 isolated bacterial strains which are designated as R1, R2, R3, R4, R5 and R6. The Performance of each of these bacterial strains is evaluated and their biodegradation rate was observed. Among the isolated strains, R3 is proved to be more efficient in degrading phenol.

Effluent sample was enriched in sterile Mineral salt agar medium (MSAM) using phenol as the sole source of carbon and energy. The sample was further treated with phenol to ensure that only phenol resistant strain would be selected. Upon enrichment with a xenobiotic compound, the natural selection of microorganisms adapted to the presence of a xenobiote has high potential for the biodegradation of the compound. Six isolates that were able to utilize phenol as sole source of carbon were obtained from the enriched population grown in MSAM medium, supplemented with phenol. These six were selected which yielded more than 80% phenol degradation and they were subjected to higher initial phenol concentration like 500 ppm and 1000 ppm. Of the strains tested, R3 showed a higher potential to degrade phenol at both 500 and 1000 ppm (Figure -3).

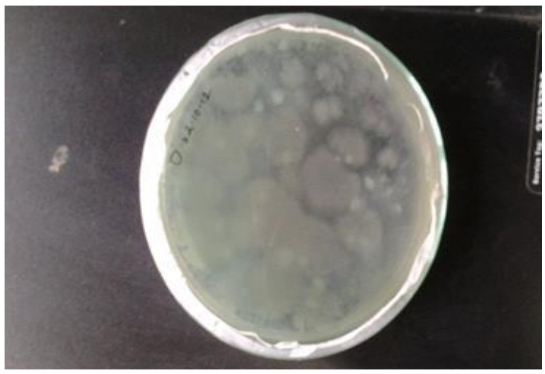

Figure 3: Isolated bacterial culture

3.4 Selection of phenol degrading microorganisms:

Pure strains capable of degrading phenol were selected by growing on MSAM medium plates by streak plate method (Figure-4).

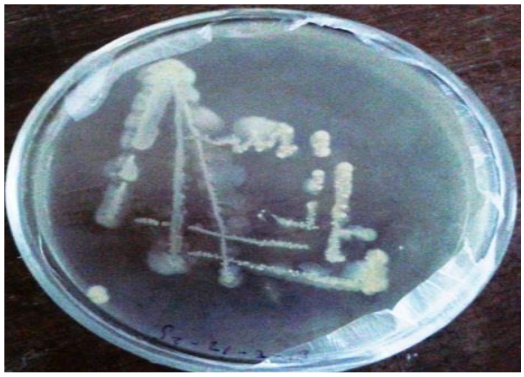

Figure 4: Streak plate method

3.5 Optimization of physiological parameters (Contact time, initial phenol Concentration, Temperature and pH):

Growth and biodegradation of any microorganism depends on various physiochemical parameters like contact time, temperature of incubator and pH of the medium and initial concentration of phenol which is used as a sole of carbon source and energy. Adsorption of any substance is also influenced by various parameters like dosage of carbon, temperature, pH, and concentration of phenol. Aim of this project, is to optimize these parameters. Study on phenol biodegradation and adsorption at different Contact time, initial concentration of phenol, temperature and pH were carried out and optimized conditions were found out.

3.5.1 Effect of Contact time:

Degradation of Phenol at various periods of time by the isolated R3 strain was studied (Figure-5). It was observed that the percentage of degradation was increased from 0 to 75 with an increase in contact of time from 24 hours to 120 hours These results show that the contact time of the medium is also an important factor with regard to degradation. The rate of degradation tends to decrease rapidly after 96 hours. Increase in exposure time resulted at lower concentrations of phenol. The toxicity threshold concentrations of phenol vary among the bacterial strains and the exposure time and indicate that bacteria could acclimate to phenol with increase in exposure time. It is suggested that for degradation of phenol is reliable and reproducible result would be best achieved within 96 h. Similar results were observed in the bacterial degradation of exposure time on phenol toxicity to refinery wastewater bacteria (Cordova-Ros et al., 2009)[11].

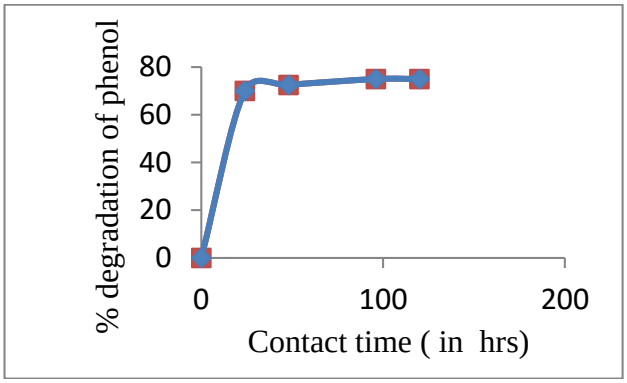

Figure 5: Effect of Contact time on % degradation of Phenol

3.5.2 Effect of initial Phenol concentration:

Experiments were conducted to study the effect of initial phenol concentration (200mg/l to 1200mg/l) on phenol removal from the solution. The results obtained are shown in Figure -6. The obtained plot shows that the percentage of degradation was decreased with an increase in initial concentration of dye. The percentage of degradation of phenol by isolated R3 strain was decreased from 77.7 to 61.7 with an increase in initial concentration from 200 to 1200 mg/l. The higher concentration of phenol inhibits nucleic acid biosynthesis and cell growth , so the effect of dye concentration on growth of organisms is an important consideration for its field application, (Khehra et al.2005)[12] suggested that the decrease in degradation efficiency might be due to increase in the toxic effect of Phenol, with increase in Phenol concentration from 200 mg/l to 1200 mg/l.

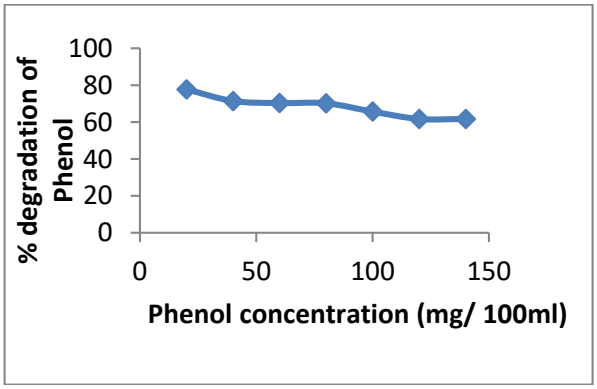

Figure 6: Effect of initial Phenol concentration on % degradation of Phenol

3.5.3 Effect of temperature:

In microorganisms the environmental temperature establishes a direct relationship with microbial activity as the microbial cell, responds to temperature changes by adaptation via biochemical or enzymatic mechanisms. The results obtained are shown in Figure-7. Experiments were conducted to determine the effect of temperature on percentage of degradation of Phenol with a constant 96 hrs time at different temperatures (25 to 42ºC) with initial phenol concentration of 200ppm. It was observed that Phenol degradation activity of the culture was found to increase with an increase in incubation temperature from 25 to 36 ºC with maximum activity attained at 36ºC (94.23% degradation). Cells may become metabolically active and capable enough to produce

the required enzymes needed for degradation. Further increase in temperature resulted in marginal reduction in degrading ability of the bacterial culture. This might have occurred due to adverse effect of high temperature on the enzymatic activities (Çetin Demet and Dönmez Gönül, 2006)[13]. Similar results were observed in the bacterial degradation of Phenolic Compounds.

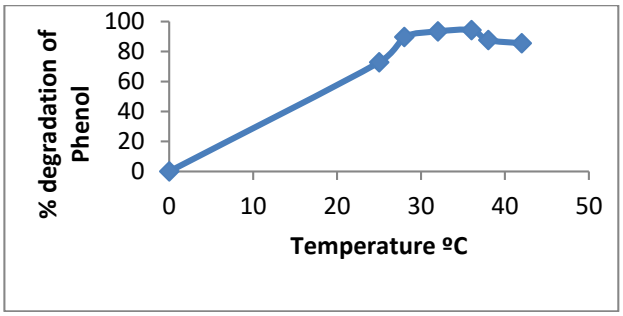

Figure 7: Effect of Temperature(ºC) on % degradation of Phenol

3.5.4 Effect of pH:

Degradation of phenol at various pH values by the strain was studied (Figure-8). It was observed that the percentage of degradation was increased from 69.23 to 97.05 with an increase in pH from 2 to 8 and decreased from 97.05 to 92.13 with an increase in pH from 8 to 12. These results show that the pH of the medium is also an important factor with regard to degradation. The rate of colour removal is higher at the optimum pH-8, and tends to decrease rapidly above pH-8. From the graph it is observed that the bacterium showed maximum degradation ability at pH-8. Degradation ability of bacteria depends on cell growth and active metabolism of culture. According to the results the organism used actively degraded the phenol at neutral alkaline conditions. By this study it can be understood that the strain actively grows at pH-8, consequently showing maximum phenol degradable ability at that pH. Similar results were observed in the bacterial degradation of various Compounds (Gurulakshmi et al.,2008) [14].

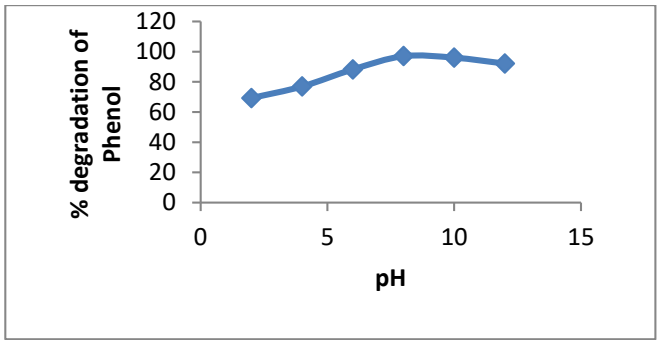

Figure 8: Effect of pH on % degradation of Phenol

3.6 Optimization of process parameters using Box-Behnken method:

The preliminary studies resulted in the following values of different process variables for maximum biodegradation of phenol (Table-1).

Table 1: Optimum parameter values obtained in preliminary studies

Parameter	Optimized value
Contact time	96hrs
Temperature	36ºC
Concentration	200ppm
pH	8

Three parameters were selected for optimization by Box-Behnken method which showed significant effect on biodegradation of phenol. They include;Temperature of incubation (X_1), Contact time (X_2) and Concentration (X_3).The optimum values used in preliminary studies have been used as the basis for selecting the mid points(zero level) in Box- Behnken method (Table-2) for further optimization.

Table 2: Coded and real values of medium components used for Box- Behnken

Independent Variables	Coded factors		
	-1	0	1
Temperature (ºC)	32	36	38
Contact time (hrs)	48	96	120
Concentration (ppm)	0	200	400

3.6.1 Experimental design for optimization:

Experiments were performed according to the Box-Behnken method given in Table-3 in order to evaluate the optimum combination of selected components in the medium.

Table 3: Result of Box- Behnken for 3 factors and comparision of experimental and predicted values of Phenol biodegradation

Run No.	Temperature (ºC)	Contact Time(hrs)	Concentration (ppm)	% Degradation of phenol (Y)		
				Experimental	Predicted	$Y_{calculated}$
1.	32(-1)	48(-1)	200(0)	-4.017	-3.858	63
2.	32(-1)	120(+1)	200(0)	-2.882	-2.848	84
3.	38(+1)	48(-1)	200(0)	-3.194	-3.229	79
4.	38(+1)	120(+1)	200(0)	-3.442	-3.601	72
5.	32(-1)	96(0)	0(-1)	-3.576	-3.751	69
6.	32(-1)	96(0)	400(+1)	-3.507	-3.524	70
7.	38(+1)	96(0)	0(-1)	-3.863	-3.845	65
8.	38(+1)	96(0)	400(+1)	-3.730	-3.554	68
9.	36(0)	48(-1)	0(-1)	-3.963	-3.947	64
10.	36(0)	48(-1)	400(+1)	-4.200	-4.341	62
11.	36(0)	120(+1)	0(-1)	-4.423	-4.281	59
12.	36(0)	120(+1)	400(+1)	-3.352	-3.369	76
13.	36(0)	96(0)	200(0)	-2.465	-2.465	91
14.	36(0)	96(0)	200(0)	-2.465	-2.465	91
15.	36(0)	96(0)	200(0)	-2.465	-2.465	91

Using the results of the experiments, the following second order regression equation, giving degradation of phenol as a function of temperature (X_1), contact time (X_2) and concentration(X_3) was obtained. Using MATLAB function 'regstats', the following coefficients are estimated.

$Y = -2.4651 - 0.030905\ X_1 + 0.15936\ X_2 + 0.12957\ X_3 - 0.3457\ X_1 X_2 - 0.016135 X_1 X3 + 0.32671\ X_2X_3 - 0.30154\ X_1^2 - 0.61735\ X_2^2 - 0.61735\ X_3^2$ (1)

The estimated coefficients along with their p-values were reported in Table-4

Table 4: Regression data for the model

Constant	Coefficient	Regression	Std. error	t- value	p- value
	β_0	-2.4651	0.10198	-24.173	2.2579e-006
X_1	β_1	-0.030905	0.062448	-0.49489	0.64165
X_2	β_2	0.15936	0.062448	2.5519	0.051145
X_3	β_3	0.12957	0.062448	2.0749	0.092663
$X_1 X_2$	β_{11}	-0.3457	0.088315	-3.9145	0.011244
$X_1 X_3$	β_{22}	0.016135	0.088315	0.18269	0.86221

$X_2 X_3$	β_{33}	0.32671	0.088315	3.6994	0.014009
X_1^2	β_{12}	-0.30154	0.091921	-3.2805	0.021943
X_2^2	β_{13}	-0.61735	0.091921	-6.7161	0.0011085
X_3^2	β_{23}	-0.61735	0.091921	-9.814	0.00018704

The coefficients of the regression model (Eq.1) calculated were listed in Table-4. The significance of the each coefficient in equation (1) was determined by student's t-test and p- values which were also listed in Table-4. The larger the magnitude of the t- value and smaller the p- value, the more significant is the corresponding coefficient. The p- values were used as a tool to check the significance of each of the coefficients, which, in turn, are necessary to understand the pattern of the mutual interactions between the test variables (Khuri and Cornell, 1996) [15]. This implies that the linear, quadratic and interaction effects of temperature, contact time and concentration were highly significant as is evident from their respective p- values.

The plot (Figure-9) showed a satisfactory correlation between the experimental and predicted values of 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.

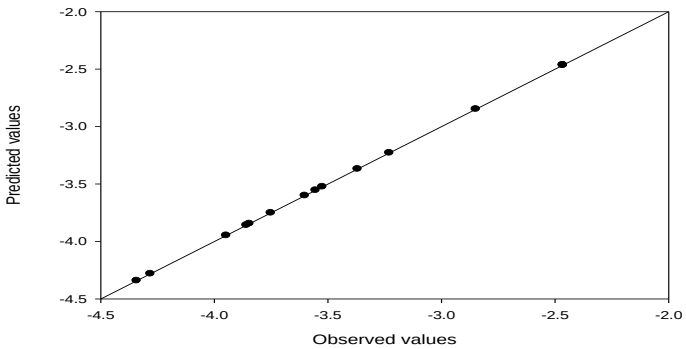

Figure 9: Comparison plot between experimental and predicted values of phenol degradation

The results of the second order response surface model fitting in the form of Analysis of Variance (ANOVA) and the value of the determination coefficient (R^2= 0.97247) were given in Table-5. 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 variance in the data about it mean. The greater the F- value 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}= 19.6249) and a very low probability value (P_{model}= 0.0022)

Table 5: ANOVA for the model

Source of variations	Degree of freedom	Sum of squares	Mean square	F value	P value
Regressions	9.0000	5.5103	0.6123	19.6249	0.0022
Residual	5.0000	0.1560	0.0312		
Total	14.0000	5.6663			

The goodness of the fit of the model was checked by the determination coefficient (R^2). The R 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= 0.97247) indicates that 97.24% of the variability in the response could be explained by the model.

3.6.2 Response Surface Plots:

The biodegradation of phenol over different combinations of independent variables was visualized through three-dimensional view of response surface plots in Figures -10 to 12. Response surface plot is a function of two factors at a time maintaining all other factors at a fixed level (zero for instance) which is more helpful in understanding both the main and interaction effects of the two factors. All the response surface plots revealed that at low and high levels of variables the degradation of

phenol was maximal, however, there existed a region where neither an increasing nor a decreasing trend in the degradation of phenol was observed. This phenomenon confirmed that there was an existence of optimum for the fermentation variables in order to maximize degradation of phenol.

3.6.2.1 Effect of temperature and contact time on biodegradation of phenol:

The interaction effect of temperature and contact time on phenol degradation in Figure-.10 clearly indicates a proper combination of, degradation of phenol. An increase temperature with contact time increased the degradation of phenol gradually but at a higher temperature with contact time the trend is reversed. The optimum for maximum phenol degradation lies near the centre point of the temperature and contact time.

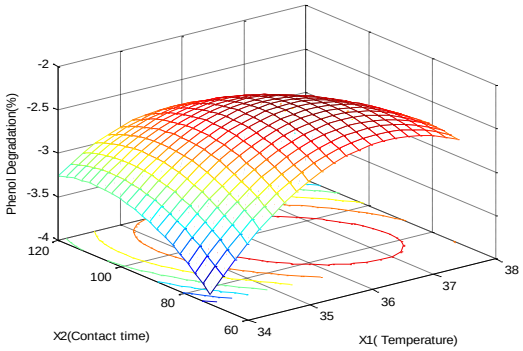

Figure10: Response surface counter plot showing the effect of temperature and contact time on degradation of phenol

3.6.2.2 Effect of temperature and concentration on biodegradation of phenol:

A similar effect on the response was observed for temperature and concentration. An increase in the temperature with concentration up to the optimum point increased the degradation of phenol to maximum level and a further increase in temperature with concentration decreased the degradation of phenol as shown in Figure-11.

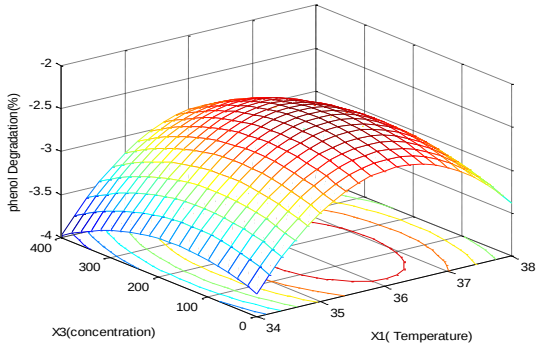

Figure 11: Response surface counter plot showing the effect of temperature and concentration on degradation of phenol

3.6.2.3 Effect of contact time and concentration on biodegradation of phenol:

The interaction effect of contact time and concentration on phenol degradation in Figurr-12 clearly indicates a proper combination of, degradation of phenol. An increase in the contact time with concentration increased the degradation of phenol gradually but at a higher contact time with concentration the trend is reversed. The optimum for maximum phenol degradation lies near the centre point of the contact time and concentration.

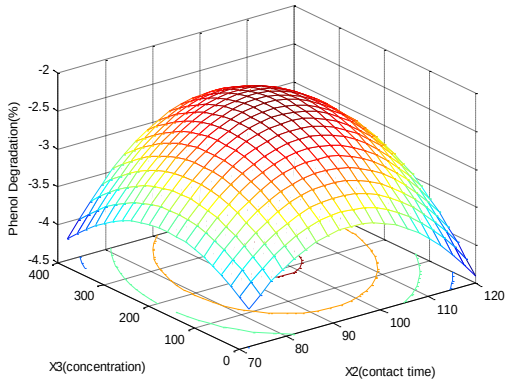

Figure 12: Response surface counter plot showing the effect of contact time and concentration on degradation of phenol

Therefore, an optimum was observed near the central value of temperature, moisture content, contact time and concentration. The optimum conditions for maximum phenol degradation were obtained at temperature of 35.6° C, contact time of 100.8 hrs, concentration of 221 ppm. An experimental phenol degradation of 0.0872 i.e. 91% was obtained at these optimum parameters. The experimental and predicted degradation of phenol at optimum conditions was shown in Table-6.

Table 6: Experimental and predicted degradation of phenol obtained from optimized parameters

Variable	Codes	Natural scale	Optimum degradation of phenol	
Temperature	-0.16513	35.66974	Experimental	Predicted

Contact time	0.20368	100.8882	0.0872	0.0865
Phenol concentration	0.10722	221.4441		

Log of optimum value of dependent variable = -2.4394
Eigen values = -0.98629 -0.6148 -0.21992

All the eigen values obtained in the present work are negative and hence, the nature of the response surface is maximum which is evident from the 3-D response plots (From Figures-9 to 11) in which the interactive effect of the 2 variables (with the third variable being fixed at its optimum value) on phenol degradation is depicted.

3.6.3 Validation of the model:

The validation was carried out in shake flasks under optimum conditions of the media predicted by the model. The experimental values for degradation of phenol were closer to the predicted values, thereby validating the model. So the validity of the equation and hence the response surface methodology are justified.

IV. CONCLUSION

Biodegradation is one of the cheapest methods with no production of hazardous by-products. This method is generally preferred due to lower costs and possibility of complete mineralization. The growth and phenol biodegradation study was carried out in MSAM with phenol as the sole carbon source and energy. Phenol degrading performance of all the strains was evaluated initially. One of the strains namely R3 was found to be highly effective for the removal of phenol. The effect of temperature, pH, and contact time and phenol concentration on the rate of phenol degradation by that particular strain was carried out. Box-Behnken design of Response Surface Methodology was used to optimize contact time, temperature and concentration for the degradation of phenol. Box-Behnken design of Response surface Methodology predicted a maximum phenol degradation of 0.0872 i.e. 91% at optimum process variables of : temperature of 35.6° C, contact time of 100.8 hrs and concentration of 221 ppm. The experimental values were closer to the predicted values and Response Surface Methodology was found to be useful tool for the optimization process.

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AUTHORS

First Author – Sridevi V, Professor, Department of Chemical Engineering, Andhra University, Visakhapatnam.
Mail; velurusridevi9848@gmail.com; vellurusridevi@yahoo.co.in
Second Author – Ranjitha M, M.Tech, Department of Chemical Engineering, Andhra University, Visakhapatnam.
Mail; mahanthiranjitha@gmail.com
Third Author – Dayana K, Associate Professor, Visakha Institute of Engineering & Technology, Visakhapatnam.
Mail; karradayana@yahoo.co.in
Fourth Author – Satya, Women Scientist, Center for Biotechnology, Department of Chemical Engineering, Andhra University, Visakhapatnam.
Mail; satya_bio1@yahoo.co.in
Fifth Author – Divyateja D, PhD Scholar, Department of Chemical Engineering, Andhra University, Visakhapatnam.
Mail; divyateja@gmail.com

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