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Isolation and Identification of Phenol Degrading Microorganism- Optimization of Process Parameters Using Box- Behnken Design Method.

Ranjitha M[1], Sridevi V[2,] , Divyateja D*[3], and Saikiran K[4].

[1] M.Tech, Department of Chemical Engineering, Andhra University, Visakhapatnam.
[2] Professor, Department of Chemical Engineering, Andhra University, Visakhapatnam.
[3*]PhD Scholar, Department of Chemical Engineering, Andhra University, Visakhapatnam.
[4]B.Tech, Department of Chemical Engineering, Andhra university, Visakhapatnam.

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, phenol concentration temperature and pH and on the rate of phenol degradation by that particular strain was carried out. Observations revealed that the rate of phenol biodegradation was significantly affected by contact time, temperature of incubation and phenol concentration. The optimal conditions for phenol removal were found to be pH of 8, temperature of 36°C, contact time of 96 hrs and concentration of phenol of 200 ppm. Statistical experimental designs, "Response surface methodology (RSM)- Box Behnken Design(BBD)" was used to optimize the process of phenol degradation by R3 strain, isolated from textile effluent. In RSM-BB method, high and low values were assigned for the three variables viz. concentration, contact time and temperature. The results of RSM-BB 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.
Keywords: Biodegradation; Identification; phenol; Optimization; BBD.

Corresponding author

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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 pollution caused by phenolic waste

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 0.001mgL^{-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 concentrations of phenol in different industrial effluents [4] are shown in the Table 1.

Table 1: Phenol concentrations in industrial effluents

Phenol concentration in industrial effluents	mg L^{-1}
Coking operations	28 – 3900
Coal processing	9 – 6800
Petrochemicals	2.8 – 1220
Pulp and paper	0.1 – 1600
Gas production	4000
Refineries	6 – 500
Pharmaceuticals	1000
Benzene manufacturing	50
Textile	100-150
Rubber	3-10
Wood preserving industry	50-953

STATISTICAL DESIGN OF EXPERIMENTS

Box–Behnken design

Box and Behnken suggested how to select points from the three-level factorial arrangement, which allows the efficient estimation of the first- and second-order coefficients of the mathematical model. These designs are, in this way, more efficient and economical then their corresponding 3*k* designs, mainly for a large number of variables [5]. In Box–Behnken designs, the experimental points are located on a hyper sphere equidistant from the central point, as exemplified for a three-factor design. Figure 1 presents the Box–

Behnken design for three-variable optimization with its 13 experimental points. This experimental design has been applied for the optimization of several chemical and physical processes; however, its application in analytical chemistry is still much smaller in comparison with central composite design. When the experimenter is relatively close to the optimum, a model that incorporates curvature is usually required to approximate the response.

$$y = \beta_0 + \sum^{k}_{i=1} \beta_i x_i + \sum^{k}_{i=1} \beta_{ii} x_i^2 + \sum\sum^{k}_{i<j} \beta_{ij} x_i x_j + \xi \qquad (1)$$

In most cases the second order model is adequate. In this section we will show how to use this fitted model to find the optimum set of operating conditions for the x' s and to characterize the nature of the response surface.

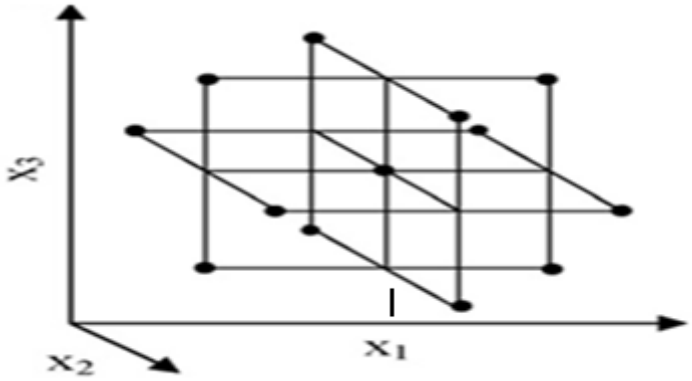

Figure 1: Box–Behnken design for the optimization of three variables

Location of the Stationary Point

We may obtain a general mathematical solution for the location of the stationary point. Writing the second-order model in matrix notation, we have

$$y = \beta_0 + \mathbf{x'\,b + x'\,Bx} \qquad (2)$$

where, **b** is a ($k \times 1$) vector of the first-order regression coefficients and **B** is a ($k \times k$) symmetric matrix whose main diagonal elements are the pure quadratic coefficients (β_{ij}) and whose off-diagonal elements are one-half the mixed quadratic coefficients (β_{ij}, $i \neq j$). The derivative of y with respect to the elements of the vector x equated to **0** is

$$\partial y/\partial x_1 = \mathbf{b} + 2\mathbf{Bx} = 0 \qquad (3)$$

The stationary point is the solution to equation 2, or

$$\mathbf{x_s} = -\,\mathbf{B}^{-1}b/2 \qquad (4)$$

Furthermore, by substituting equation 4 into equation 2, we can find the predicted response at the stationary point as

$$Y_s = \beta_0 + x'_s b/2 \qquad (5)$$

The Eigen values of matrix **B** give an indication about the nature of response surface. For a function representing maximum surface, all the Eigen values are negative, whereas they are positive for a function with minimum. If Eigen values have different signs (i.e. some positive, and some negative), the surface is saddle for which a minimum or maximum cannot be specified.

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MATERIALS AND METHODS

Sample Collection

The effluent samples were collected from Textile industry [6].

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 [7]. 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.

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 [8]. 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.

Steam Distillation

Figure 2: Steam Distillation Setup **Figure 3: Optimization of parameters**

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 hot water in the boiling flask that will generate steam and thus will provide an internal source of steam. Add to the boiling flask at least three times as much as water as sample[12]. Do not fill the flask much more than half full. Periodically, add more hot water as needed, when the water boils and turns to steam, it also leaves the flask, carrying the product. Often times, while a distillation is in progress, steam may condense on the sides of the biomass flask and the distillation arm before it makes its way into the condenser. This is simply a property of a distillation system that contains a large surface area

of glass. Hot steam tends to condense back into water as soon as it can. To minimize the amount of steam that condenses too soon and thus drips back into the boiling flask, insulation like cloth or foam may be wrapped around the biomass flask and distillation arm. Additionally, make sure that the plant material in the biomass flask is not packed too tightly. In any case, some steam will inevitably condense and drip back into the boiling flask during operation. It is typical for some of the boiling water to remain (or drip back down) in the boiling flask for the duration of the distillation. This water may also take on a yellow to brown color as water soluble components of the material are released from the plant during distillation. Monitoring the progress of the distillation is a skill that requires practice, patience, and research into the specifics of the plant material being used. Many people prefer to end their distillation when the hydrosol water dripping from the end of the condenser loses the pleasant aromas prominent at the beginning stages of the process. Again, it is not unusual that some water will remain in the boiling flask when it is time to end a successful distillation. The distillation set up was shown in the Figure 2.

Isolation and Screening of Phenol degrading microorganisms

The process consists of the following steps

Media Preparation ⟶ Sterilization ⟶ Culturing ⟶ Inoculation

Media preparation

MSAM and MSM composition was added in 500 ml of distilled water in a conical flask.

Media Used For Isolation:

The following media was used for isolation of potential [9]. The composition of MSAM medium was shown in Table 2[10].

Table 2: Composition of Mineral salt Agar medium

Chemical Composition	g/L
Sodium nitrate	2.0
Magnesium Sulfate	0.5
Potassium Chloride	0.5
Ferric sulfate	0.01
Pottasium Dihydrogen Phosphate	1.5
Dipotassium Hydrogen Phosphate	1.7
Agar	15
Yeast Extract	2.0
Sodium Sulfate	3.0
Sodium Chloride	0.5
Calcium Chloride	0.5
Ammonium Chloride	0.5
Nutrient Agar	0.24
P^H	7.2

Maintenance Medium

The composition of maintenance medium was shown in Table 3[11].

Table 3: Composition of mineral salt medium

Chemical Composition	g/L
KH_2PO_4	1.5
K_2HPO_4	0.5
NH_4Cl	0.5
$CaCl_2$	0.02

$MgSO_4$. $7H_2O$	0.2
NaCl	0.5
Na_2SO_4	3.0
Yeast Extract	2.0
Nutrient Agar	2.0

Heat Sterilization

It is the process, in which foreign contamination is removed by means of heat, in lab Auto-clave was used for heat sterilization. After the preparation of MSAM, it was kept in auto-clave. A pressure of 15-20 psi and temperature of 120-121^oC was maintained in autoclave. It was sterilized for 15-20 minutes and then cooled at room temperature.

Culturing

Culturing is a process in which a nutrient media is provided for micro-organism growth [15]; the media is called culture media.

Culture media: Mineral salt Agar media

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 37^0C for about a week. Regular observations were made.

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)[16]. 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.

Maintenance of Phenol resistant isolates

Isolates transferred were grown on MSAM phenol agar slants and sub-cultured [17]. 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.

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 [13]. After 48 hours, the Cell density was determined spectrophotometrically by measuring turbidity at 600nm.

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 [18]. Preliminary degrading studies were carried out with addition of isolated strain containing different concentrations of phenol [14], different periods of time, different pH values and different temperature conditions [19]. 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.

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 [24].

Preliminary Studies for determining Optimum conditions:

Selected newly isolated bacterial strains were grown in the nutrient broth by incubation at overnight, at 37^0C 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 following physical and chemical parameters of phenol degradation by selected newly isolated bacteria was carried out with MSM medium (Figure 3). Phenol degradation experiments were carried out in 250mL shake flask containing 100mL of MSM medium with 200mgL^{-l} of phenol as sole carbon source [20]. The medium was inoculated with R3 strain to initiate the cultivation and degradation of phenol and centrifuged at 1400rpm for 1 hr [21]. The supernatant was withdrawn at regular intervals (24hr) and analyzed for cell growth and phenol concentration.

Effect of contact time

Effect of Contact time on degradation was studied at different period of times varying from 24h to 120h. All the prepared media were autoclaved and carefully inoculated with R3 strain culture using a micropipette and incubated in an orbital shaking incubator at 37°C and 120rpm. Sampling was performed for every 24 hours and the data obtained through spectrophotometric analysis were collected and converted into percentage degradation using the 4- Amino antipyrine method.

Effect of initial phenol concentration

Degradation activity of the culture of *R3 Strain* was studied at different phenol concentrations varying from 200mg/l to 1400mg/l. All the prepared media were autoclaved and carefully inoculated with R3 strain culture using a micropipette and incubated in an orbital shaking incubator at 37°C and 120rpm. Sampling was performed for every 24 hours and the data obtained through spectrophotometric analysis were collected and converted into percentage degradation using the 4- Amino antipyrine method.

Effect of pH

Effect of pH on degradation was studied at different pH varying from 4 to 12. pH of the media were adjusted using 0.1N HCL and 0.1N NaOH. All the prepared media were autoclaved and carefully inoculated with R3 strain culture using a micropipette and incubated in an orbital shaking incubator at 37°C and 120rpm. Sampling was performed for every 24 hours and the data obtained through spectrophotometric analysis were collected and converted into percentage degradation using the 4- Amino antipyrine method.

Effect of temperature

Effect of temperature on degradation was studied at four different temperatures (25, 28, 32, 36, 38 and 42). All the prepared media were autoclaved and carefully inoculated with R3 strain culture using a micropipette and incubated in an orbital shaking incubator at selected temperatures at 120 rpm. Sampling was performed for every 24 hours and the data obtained through spectrophotometric analysis were collected and converted into percentage degradation using the 4- Amino antipyrine method.

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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.

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

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[22]. Range fixation for process variables is quite crucial when carrying experiments according to any statistical design of experiment, otherwise once the experimentation is over[23], the optimal conditions obtained by RSM might not be found within the chosen range.

RESULTS AND DISCUSSION

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 4).

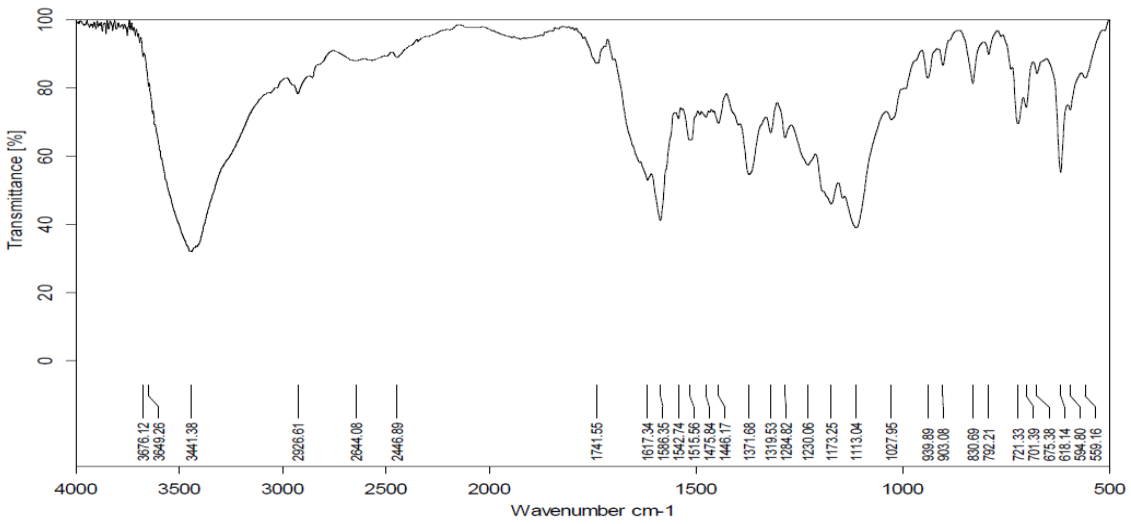

Figure 4: FTIR Spectrum of the sample

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.

Isolation and characterization of bacterial strains

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. 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. 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. Pure strains capable of degrading phenol were selected by growing on MSAM medium plates by streak plate method.

Optimization of physiological parameters (Contact time, Concentration Temperature and pH)

Growth and biodegradation of any microorganism depends on various physiochemical Parameters. Study on phenol biodegradation and adsorption at different Contact time, concentration of phenol, temperature and pH and are carried out and optimize conditions are found out.

Effect of Contact time

Degradation of Phenol at various periods of time by the isolated R3 strain was studied. It was observed that the percentage of degradation was increased from 0 to 75 with an increase in contact of time from 24 hours. The rate of degradation tends to decrease rapidly after 96 hours. At sufficient concentrations, exposure of these bacterial cells to phenol resulted in efficient degrading activity. 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.

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 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, 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.

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. 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 42°C) 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.

Effect of pH

Degradation of phenol at various pH values by the strain was studied. 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.

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 4). 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 5) for further optimization.

Table 4: Optimum parameter values obtained in preliminary studies

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

Table 5: 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

Experimental design for optimization:

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

$$Y = -2.4651 - 0.030905\,X_1 + 0.15936\,X_2 + 0.12957\,X_3 - -0.3457\,X_1X_2 - 0.016135\,X_1X3 + 0.32671\,X_2X_3 - -0.30154\,X_1^2 - 0.61735\,X_2^2 - 0.61735\,X_3^2 \quad (6)$$

The estimated coefficients along with their p-values were reported in Table 6

Table 6: Regression data for the model

	Coefficient	Regression	Std. error	t- value	p- value
constant	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_1X_2	β_{11}	-0.3457	0.088315	-3.9145	0.011244
X_1X_3	β_{22}	0.016135	0.088315	0.18269	0.86221
X_2X_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 (6) calculated were listed in Table 6. The significance of the each coefficient in equation (6) was determined by student's t-test and p- values which were also listed in Table 6. 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 variable. 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 5) 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.

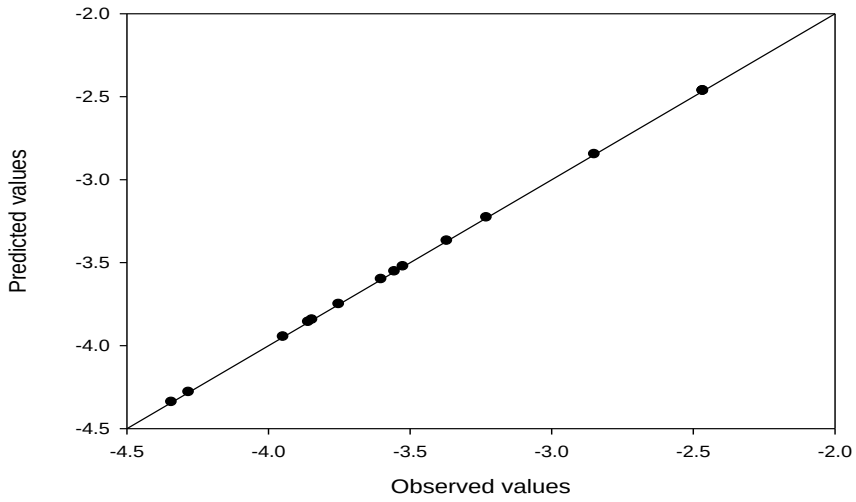

Figure 5: Comparison plot between experimental and predicted values of phenol degradation

Response Surface Plots

The biodegradation of phenol over different combinations of independent variables was visualized through three- dimensional view of response surface plots in Figure 6 to 8. 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.

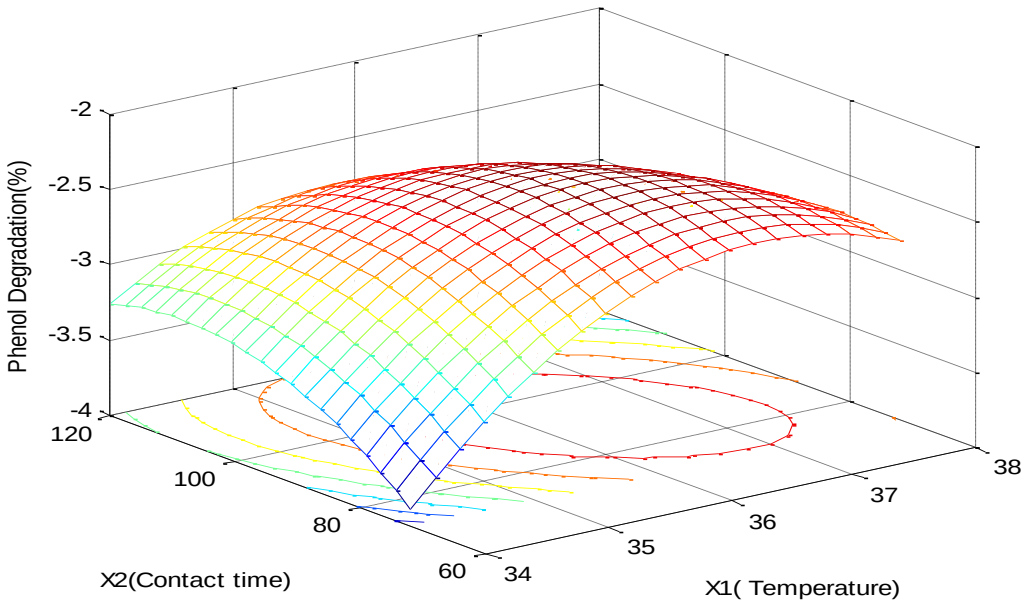

Figure 6: Response surface counter plot showing the effect of temperature and contact time on degradation of phenol

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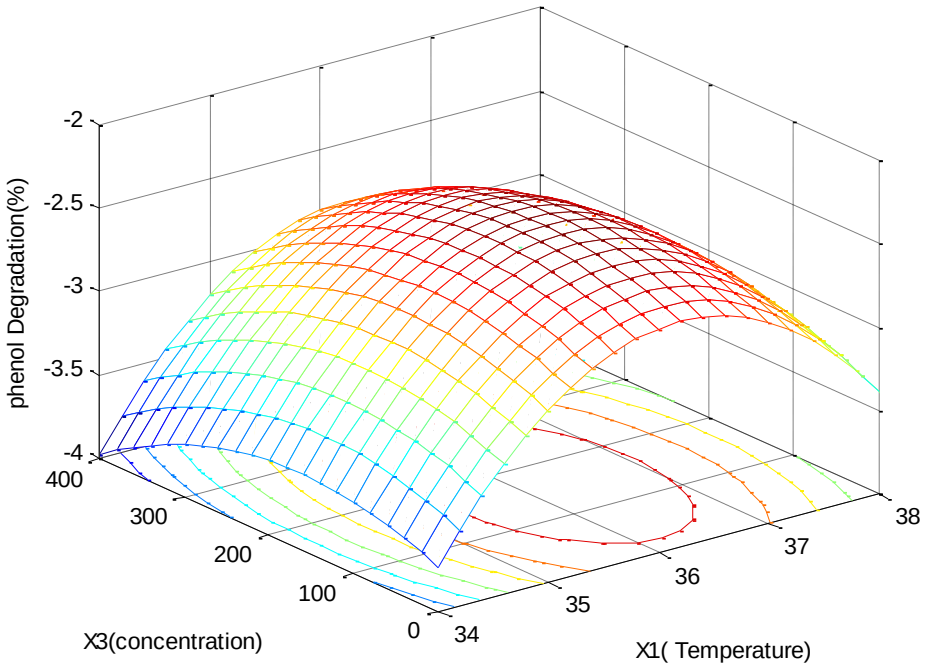

Figure 7: Response surface counter plot showing the effect of temperature and concentration on degradation of phenol

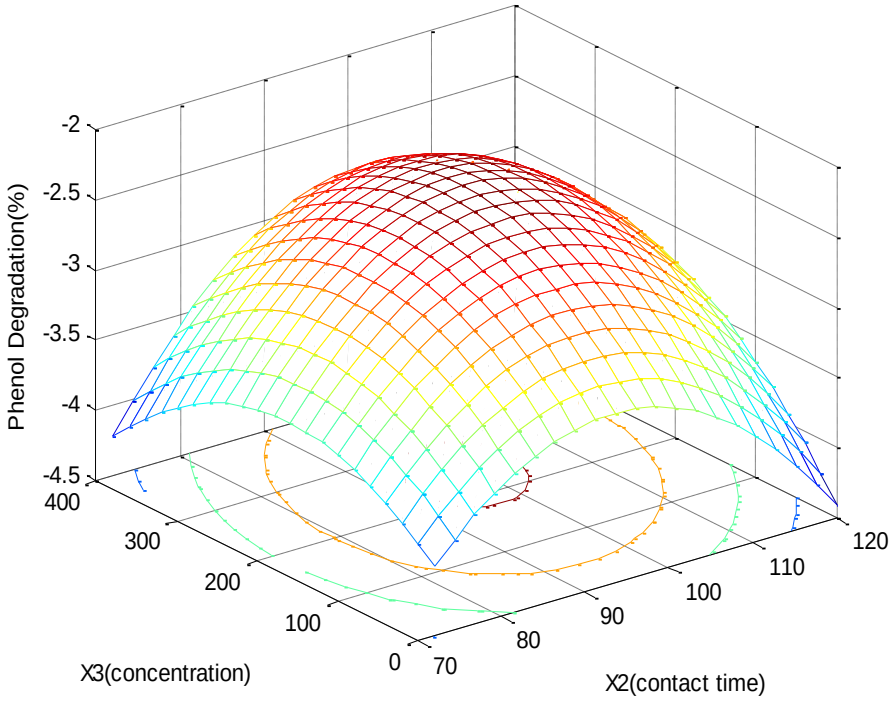

Figure 8: Response surface counter plot showing the effect of contact time and concentration on degradation of phenol

Effect of temperature and contact time on biodegradation of phenol:

The interaction effect of temperature and contact time on phenol degradation in Figure 6, 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.

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 7.

Effect of contact time and concentration on biodegradation of phenol

The interaction effect of contact time and concentration on phenol degradation in Figure 8, 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.

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 7.

Table 7: 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		
Phenol concentration	0.10722	221.4441	0.0872	0.0865

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 Figure 6 to 8) in which the interactive effect of the 2 variables (with the third variable being fixed at its optimum value) on phenol degradation is depicted.

SUMMARY

All the bacterial isolates, isolated from the phenol enriched effluent, which were capable to grow on phenol as sole carbon source were screened for their ability to grow and degrade phenol at 200 ppm concentration. Out of these, the R3 has shown a higher potential to degrade phenol at 200 ppm which ultimately led to higher biomass production by the strain, hence, selected for further degradation studies. The various process parameters like contact time, temperature, initial phenol concentration and pH were optimized step-by-step in preliminary experiments. Effect of pH on phenol degradation by individual microorganism shows the rate of phenol degradation by R3 strain is maximum at pH 8. Influence of incubation temperature suggests the rate of phenol degradation by R3 strain is maximum at an optimum temperature 36°C. Under optimized condition the microorganism is able to degrade 200 ppm of phenol completely in 96 hours. With increase in the concentration of the phenol in the medium, the specific growth of the microorganism decreases which owes to the toxic nature of the substrate. The Box- Behnken method was conducted in the optimum vicinity to locate the true optimum concentrations for the 3 factors which showed significant effect on phenol degradation - temperature (X1), contact time (X2) and concentration (X3). Fifteen experiments were conducted according to Box- Behnken and degradation of phenol was measured in each case. A second order quadratic equation was obtained. The second-order polynomial coefficients were

calculated to determine the role of each variable, their interactions and statistical analysis to obtain predicted degradation of phenol. Using the 'MATLAB function regstats ', the data obtained was analyzed and response surface plots were constructed which indicated the possibility of enhancement in the production of degradation of phenol. Statistical analysis of the model was performed to evaluate the analysis of variance. The predicted optimum levels were as follows: temperature of 35.6° C, contact time of 100.8 hrs and concentration of 221 ppm. The experimental yield of phenol degradation at optimum conditions was 0.0872 i.e. 91%, which was in close agreement with the value predicted by the model, 0.0865. The value of regression coefficient R2 = 0.97247 indicates that 97.24% of the variability in the response could be explained by the model.

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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