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Statistical optimization of medium components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*

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Abstract

In this work, a four-level Box–Behnken factorial design was employed combining with response surface methodology (RSM) to optimize the medium composition for the degradation of phenol by *pseudomonas putida* (*ATCC 31800*). A mathematical model was then developed to show the effect of each medium composition and their interactions on the biodegradation of phenol. Response surface method was using four levels like glucose, yeast extract, ammonium sulfate and sodium chloride, which also enabled the identification of significant effects of interactions for the batch studies. The biodegradation of phenol on *Pseudomonas putida* (*ATCC 31800*) was determined to be pH-dependent and the maximum degradation capacity of microorganism at 30 °C when the phenol concentration was 0.2 g/L and the pH of the solution was 7.0. Second order polynomial regression model was used for analysis of the experiment. Cubic and quadratic terms were incorporated into the regression model through variable selection procedures. The experimental values are in good agreement with predicted values and the correlation coefficient was found to be 0.9980.

Keywords: Biodegradation of phenol; *Pseudomonas putida* (*ATCC 31800*); Box–Behnken design; Correlation coefficient

1. Introduction

Petroleum based products have led to large prevalence of phenolic compounds in effluents and waste materials. Coking plants, oil refineries, and several chemical industries during the processing of resins, plastics, dyes, pharmaceuticals and pesticides generate phenols. Phenol can cause body disorders like allergic dermatitis, skin irritation, cancer and genetic disorders like mutation. Studies have shown phenols to be carcinogenic and to cause taste and odour problem in drinking water. Thus phenols are included in the U.S. Environmental Protection Agency list of priority pollutants in the super found program [1,2]. Phenolic compounds and most aromatic hydrocarbons have a benzene ring in their basic structures. The benzene molecule and benzene derivatives are less reactive and more stable than single-chained (aliphatic) compounds because of the large amount of energy required to break apart a cyclic structure [3–5].

The conventional methods for treating phenol-containing wastewater use activated carbon [6]. This accounts for the renewed interest in the use of absorbent for the complex treatment of wastewater containing phenols. But the use of adsorbent is limited because of its high cost. Many xenobiotic compounds thought to be too toxic for microorganisms to degrade can be degraded under aerobic, anaerobic (or) anoxic conditions [7]. Biodegradation is versatile, inexpensive and can potentially turn a toxic material into harmless products. If properly designed and operated, biological processes can realize total oxidation of organic matter so that there can be no sludge's that must be eradicated as a result of treatment. Removal of phenol from such wastewaters can be achieved through aerobic biodegradation in well-run activated sludge plants [8]. A *Pseudomonas sp.* strain capable of degrading pentachlorophenol was isolated around tannery soil and characterized as *Pseltdomonas aeriginosa. Cyanobacteria* are in more advantageous position than heterotrophic bacteria because of its trophic inde-

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pendence for nitrogen as well as carbon [7]. Phenol, the toxic constituent of several industrial effluents, was found to be effectively removed and degraded by the marine *Cyanobacterium phormidium valderianum* [9–11]. Although bacteria are most likely to be responsible for aerobic breakdown of phenol in activated sludge, fungi, including *Trichosporon cutaneum* and *Candida tropicalis* cells are also capable of utilizing phenol as the major carbon source [12]. A pure culture of *Pseudonionas putida* was grown in both a batch and continuous culture using phenol as the limiting substrate. The traditional kinetic model based on the Haldane, Monod equations may be inadequate for describing the dynamics of phenol degrading systems [13,14]. The microbial degradation of phenol by pure and mixed cultures of *P. putida* is able to remove phenol from wastewaters down to levels of 1–2 ppm in a single stage system. Using immobilized cell is one of the approaches for incorporating bacterial biomass into an engineering process. The advantages of the process based on immobilized biomass include the following; enhancing microbial cell stability, allowing continuous process operation and avoiding the biomass-liquid separation requirement. Physical entrapment of organisms inside a polymeric matrix is one of the most widely used techniques for whole-cell immobilization [15–17]. Polyacrylamide silica gels have been the most extensively used immobilization materials for laboratory research studies [18–20].

Naturally occurring carbon sources can have a significant impact on the ability of microbial communities to degrade pollutants. In general, adaptation to increase (or) decrease in concentration of glucose, yeast extract, $(NH_4)_2SO_4$ and NaCl enhanced the ability to degrade to the phenols. The pollutants were qualitatively the natural substances of phenol, whereas the magnitude of the enhancement varied and the basic trends were very similar. It seems likely that the results depend on the substrates used and does not have any particular biochemical relationship between a single class of phenol. The process of co-metabolism is an important example of the influence that a readily degradable carbon substrate can have on the biodegradation of pollutants. In co-metabolic process, a compound which is normally stable and incapable of supporting bacterial growth can be partially degraded, but not used for carbon (or) energy, when a readily degradable secondary source of carbon is available.

In the present investigation *Pseudomonas spp.* were screened and *P. putida* (*ATCC 31800*), which degraded phenol in a short period of time and had higher tolerance limit, was selected for the adaptation. Therefore, it was thought worthwhile to study the effect of different media and to consider various environmental factors like, glucose, yeast extract, $(NH_4)_2SO_4$ and NaCl that affect the biodegradation of phenol. We have attempted to optimize the cultivation process for the biodegradation of phenol by *P. putida* (*ATCC 31800*). The optimization of the medium for degradation has been performed for the response surface methodology, which is a software-supported experimental design technique that enables logical inputs in terms of interaction of media components and quantities and the relationship between these independent variables and responses.

2. Design of experiments

Response surface methodology is an empirical modelization technique derived to the evaluation of the relationship of a set of controlled experimental factors and observed results. It requires a prior knowledge of the process to achieve statistical model [21–24]. Basically this optimization process involves three major steps, which are, performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the adequacy of the model.

$$Y = f(X_1, X_2, X_3, X_4 \ldots X_k) \tag{1}$$

The true relationship between Y and X_k may be complicated and, in most cases, it is unknown, however, a second-degree quadratic polynomial can be used to represent the function in the range of interest;

$$Y = R_o + \sum_{i=1}^{k} R_i X_i + \sum_{i=1}^{k} R_{ii} X_i^2 + \sum_{i=1,i<j}^{k-1} \sum_{j=2}^{k} R_{ij} X_i X_j + \varepsilon \tag{2}$$

where $X_1, X_2, X_3, X_4, \ldots, X_k$ are the input variables which affect the response Y, R_0, R_i, R_{ii} and R_{ij} ($i = 1-k$, $j = 1-k$) are the known parameters, ε is the random error. A second-order model is designed such that variance of Y is constant for all points equidistant from the center of the design;

$$X_i = \left(\frac{X_i - X_o}{\Delta X_i} \right) \tag{3}$$

where X_i is the coded value, X_o is the actual value at the center point and ΔX_i is the step change value. The parameters and their values (in brackets) were four levels, like glucose (0.25, 0.5, 0.75 mg/L), yeast extract (0.05, 0.15, 0.25 g/L), ammonium sulfate (1, 3, 5 g/L), sodium chloride (0.25, 0.5, 0.75 g/L), at constant phenol concentration 0.2 g/L, pH 7.0 and temperature-30 °C. This also enabled the identification of significant effects of interactions for the batch studies. In system involving four significant independent variables X_1, X_2, X_3, and X_4, the mathematical relationship of the response of these variables can be approximated by quadratic (second degree) polynomial equation;

$$\begin{aligned} Y = {} & b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4 + b_{11} X_1^2 + b_{22} X_2^2 \\ & + b_{33} X_3^3 + b_{44} X_4^2 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{14} X_1 X_4 \\ & + b_{23} X_2 X_3 + b_{24} X_2 X_4 + b_{34} X_3 X_4 \end{aligned} \tag{4}$$

where Y is the predicted value, b_o is the constant, X_1 is the glucose, X_2 is the yeast extract, X_3 is $(NH_4)_2SO_4$, X_4 is NaCl, b_1, b_2, b_3 and b_4 are linear coefficients, b_{12}, b_{13}, b_{14}, b_{23}, b_{24} and b_{34} are cross product coefficients and b_{11}, b_{22}, b_{33} and b_{44} are quadratic coefficients. These parameters were chosen as carbon and notrogen sources have designed X_1, X_2, X_3, and X_4, respectively. The low middle and high levels of each variable were designated as −1, 0, and +1 respectively, as given in Table 1. A total of 29 treatments were necessary to estimate the coefficients of the

Table 1
The Box–Behnken design for the four independent variables on biodegradation of phenol in actual and predicted values

Run no.	Glucose (g/L) X_1	Yeast extract (g/L) X_2	$(NH4)_2SO_4$ (g/L) X_3	NaCl (g/L) X_4	Experimental value (%)	Predicted value (%)
1	0.25(−1)	0.05(−1)	3.0(0)	0.50(0)	87.66	87.66
2	0.75(+1)	0.05(−1)	3.0(0)	0.50(0)	76.91	76.91
3	0.25(−1)	0.25(+1)	3.0(0)	0.50(0)	92.00	91.49
4	0.50(0)	0.25(+1)	3.0(0)	0.50(0)	92.80	92.54
5	0.50(0)	0.15(0)	1.0(−1)	0.25(−1)	93.38	93.47
6	0.50(0)	0.15(0)	5.0(+1)	0.25(−1)	95.62	95.93
7	0.50(0)	0.15(0)	1.0(−1)	0.75(+1)	97.95	98.05
8	0.25(−1)	0.15(0)	5.0(+1)	0.75(+1)	84.21	84.52
9	0.75(+1)	0.15(0)	3.0(0)	0.25(−1)	95.96	96.00
10	0.75(+1)	0.15(0)	3.0(0)	0.25(−1)	91.46	91.06
11	0.75(+1)	0.15(0)	3.0(0)	0.75(+1)	92.46	92.50
12	0.50(0)	0.05(−1)	3.0(0)	0.75(+1)	88.13	87.73
13	0.50(0)	0.25(+1)	1.0(−1)	0.50(0)	89.84	89.27
14	0.50(0)	0.50(−1)	1.0(−1)	0.50(0)	92.90	92.91
15	0.50(0)	0.25(+1)	5.0(+1)	0.50(0)	78.01	77.65
16	0.25(−1)	0.15(0)	5.0(+1)	0.50(0)	95.66	96.45
17	0.75(+1)	0.15(0)	1.0(−1)	0.50(0)	96.23	95.76
18	0.25(−1)	0.15(0)	1.0(−1)	0.50(0)	98.00	98.28
19	0.75(+1)	0.15(0)	5.0(+1)	0.50(0)	97.92	97.59
20	0.50(0)	0.15(0)	5.0(+1)	0.50(0)	85.50	85.36
21	0.50(0)	0.05(−1)	3.0(0)	0.25(−1)	93.22	92.91
22	0.50(0)	0.25(+1)	3.0(0)	0.25(−1)	92.03	92.30
23	0.50(0)	0.05(−1)	3.0(0)	0.75(+1)	79.47	79.15
24	0.50(0)	0.25(+1)	3.0(0)	0.75(+1)	88.94	89.21
25	0.50(0)	0.15(0)	3.0(0)	0.50(0)	94.10	94.10
26	0.50(0)	0.15(0)	3.0(0)	0.50(0)	94.10	94.10
27	0.50(0)	0.15(0)	3.0(0)	0.50(0)	94.10	94.10
28	0.50(0)	0.15(0)	3.0(0)	0.50(0)	94.10	94.10
29	0.50(0)	0.15(0)	3.0(0)	0.50(0)	94.10	94.10

model using multiple linear regressions. The design of experiments was carried out for analysis using the design expert by Stat Ease lnc, Statistics Made Easy, Minneapolis, MN Version .7).

3. Material and methods

3.1. Inocula

P. putida (*ATCC 31800*) was procured from culture collection in Bioresources Collection and Research Center, Food Industry Research and Development Institute, Taiwan. The bacteria were grown and maintained on beef extract: 1.0 g/L, yeast extract: 2.0 g/L, peptone: 5.0 g/L, NaCl: 5.0 g/L and agar: 20 g/L. The medium was adjusted to pH 7.0 by 1N NaOH. It was stored at 4 °C ± 1 °C until for the use. Phenol, 4-amino antipyrine and all other chemicals used were obtained from Sigma-Aldrich chemical company in USA.

3.2. Optimization studies

The studies were carried out in the following media: glucose, (medium-1): (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 g/L); yeast extract (medium-II): (0.05, 0.1, 0.15, 0.2, 0.25, 0.3 g/L); ammonium sulphate, (medium-III): (1, 2, 3, 4, 5, 6 g/L) and NaCl, (medium-IV): (0.25, 0.5, 1.5, 2.0, 2.5 .0 g/L) at constant 0.6 g/L phenol concentration with minimal medium by P. *putida* (*ATCC 31800*) inoculated at 30 °C, pH 7.0 and at 180 rpm in orbit shaker for 48 h. The above variables namely glucose, yeast extract, ammonium sulphate and sodium chloride, were varied and the maximum degradation of phenol was optimized.

3.3. Box–Behnken design experiments

Minimal medium used composed of K_2HPO_4: 1.5 g/L; KH_2PO_4: 0.5 g/L; $(NH_4)_2$ SO_4:3.0 g/L; NaCl: 0.5 g/L; $MgSO_4$: 0.5 g/L; Calcium chloride: 0.02 g/L; $FeSO_4$: 0.02 g/L and phenol: 0.600 g/L. Minimal medium with added carbon sources like glucose (X_1), yeast extract (X_2), $(NH_4)_2SO_4$ (X_3) and NaCl (X_4) at different concentrations of above nutrient are designed (Design expert 7.0) as shown in Table 1. The design experiments (from Table 1) were carried out in conical flasks containing minimal medium and inoculated with P. *putida* (*ATCC 31800*) at 30 °C, pH 7.0 and at 180 rpm in shaker for 48 h. After 48 h, the amount of phenol degraded was calculated by taking samples.

3.4. Phenol determination

Phenol was determined quantitatively by the spectrophotometric method (Beckmann DU 40 Model) using 4-amino antipyrine as the colour reagent (λ_{max}: 500 nm) according to standard methods of analysis [25].

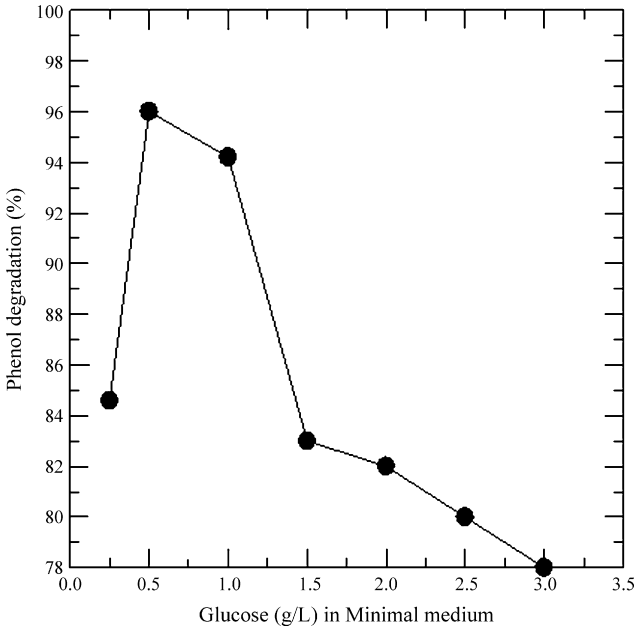

Fig. 1. Optimum concentration of glucose for phenol degradation in minimal medium.

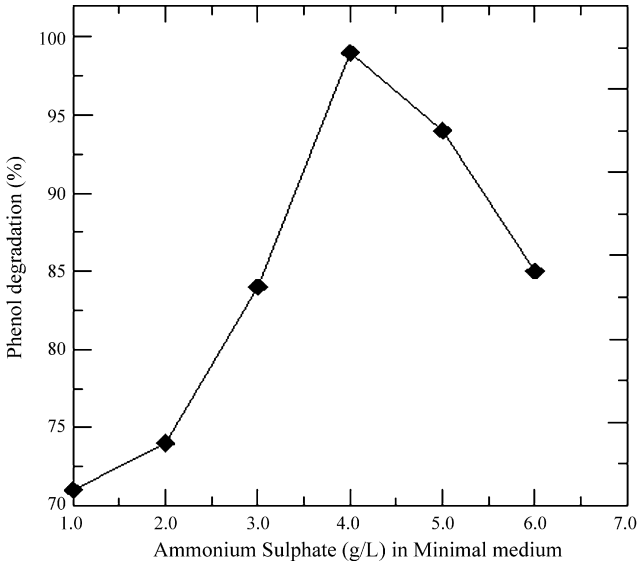

Fig. 3. Optimum concentration of ammonium sulphate for phenol degradation in minimal medium.

4. Results and Discussion

P. putida (*ATCC 31800*) was inoculated into minimal medium containing different concentrations of glucose, yeast extract, ammonium sulphate and sodium chloride. The optimum concentration as shown in Figs. 1 to 4 shows maximum percentage of phenol degradation. The optimum concentration of (Figs. 1 and 4), glucose, (0.25, 0.5, 0.75 g/L), yeast extract, (0.05, 0.15, 0.25 g/L), $(NH_4)_2SO_4$, (1, 3, 5 g/L) and NaCI (0.25, 0.5, 0.75 g/L) were determined using Box–Behnken design of experiments. The regression equation obtained after analysis of variance gives the level of biodegradation of phenol as a function of the different concentrations of glucose, yeast extract, $(NH_4)_2SO_4$ and NaCl. A regression model containing four linear (X_1, X_2, X_3, X_4), four quadratic (X_1^2, X_2^2, X_3^2, X_4^2) and six interaction($X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4$) terms plus one block term was employed by using DESIGN EXPERT version (5.7.0.1.1997). The experimental results were analyzed through RSM to obtain in empirical model for the best response. The results of theoretically predicted response are shown in Table 1. The estimated response seems to have a functional relationship only in a local region (or) near the central points of the model. The quadratic model was used to explain the mathematical relationship between the independent variable and dependent

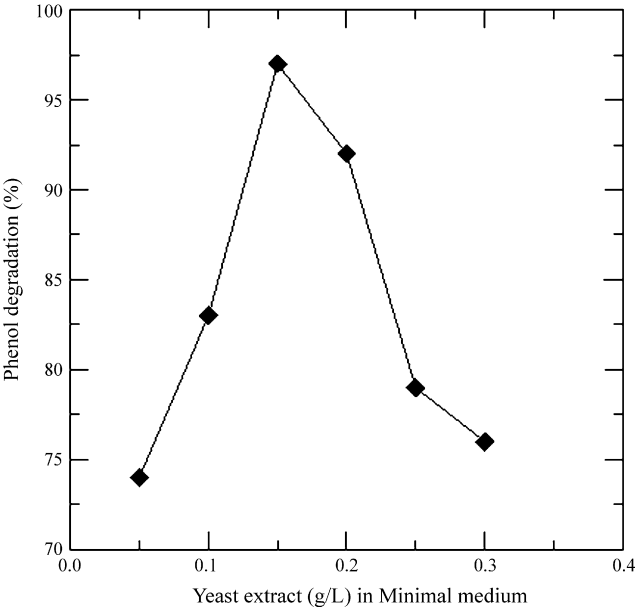

Fig. 2. Optimum concentration of yeast extract for phenol degradation in minimal medium.

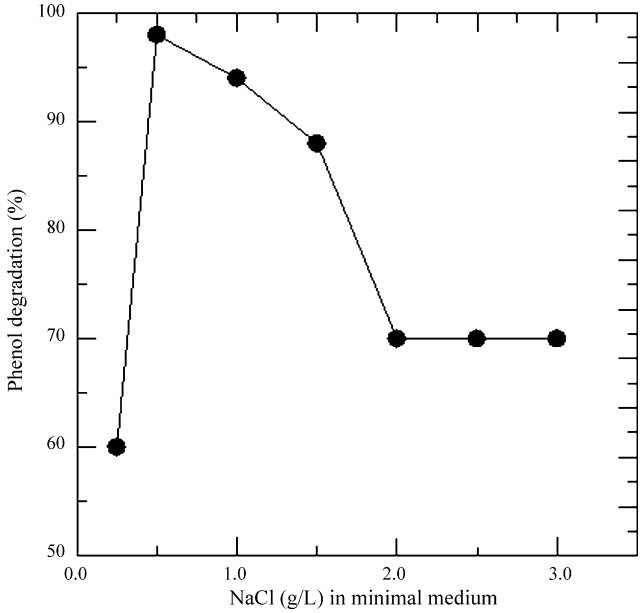

Fig. 4. Optimum concentration of sodium chloride for phenol degradation in minimal medium.

Table 2
Regression analysis for the biodegradation of phenol by *P.putida* for quadratic response surface model fitting (ANOVA)

Source	Coefficient value	Mean square	*F*-value	Probability > *F* value
b_0	94.24	0.41	–	–
b_1	−2.83	0.26	−9.20	<0.0001*
b_2	4.86	0.026	18.44	<0.0001*
b_3	−2.77	0.26	−10.50	<0.0001*
b_4	−0.58	0.36	−1.62	0.1277
b_{11}	−0.58	0.36	−1.62	0.1277
b_{22}	−6.51	0.36	−18.15	<0.0001*
b_{33}	0.59	0.36	1.64	0.1277
b_{44}	−1.84	0.36	−5.12	<0.0001*
$b_1\ b_2$	2.95	0.46	6.46	<0.0001*
$b_1\ b_3$	−3.69	0.46	−8.07	<0.0001*
$b_1\ b_4$	−0.43	0.46	0.093	0.9272
$b_2\ b_3$	3.04	0.46	6.66	<0.0001*
$b_2\ b_4$	0.17	0.46	0.37	0.7193
$b_3\ b_4$	−4.00	0.46	−8.75	<0.0001*
Model	976.06	69.72	82.54	<0.0001*
Residual	11.68	0.83		
Lack of fit	3.19	0.32	0.15	<0.9929
Pure error	8.49	2.12	–	–
Correlation total	987.75	*R*-squared = 0.9980	Adj-*R* squared = 0.9959	–

* Values of "probability > *F*" less than 0.0500 indicate model terms are significant.

responses. The mathematical expression of relationship to the phenol degradation with variables like glucose, yeast extract, ammonium sulfate and sodium chloride are shown below as in terms of coded factors. All terms regardless of their significance are included in the following equation.

$$Y = 94.24 - 2.83X_1 + 4.86X_2 - 2.77X_3 - 0.58X_4 - 0.58X_1^2 + 6.51X_2^2 + 0.59X_3^3 - 1.84X_4^2 + 2.95X_1X_2 - 3.69X_1X_3 - 0.43X_1X_4 + 3.04X_2X_3 + 0.17X_2X_4 - 0.4.0X_3X_4 \quad (5)$$

The results of analysis of variance (ANOVA) are shown in Table 2 which indicates that the predictability of the model is at 99% confidence interval. The predicted response fit the well with those of the experimentally obtained response. A coefficient of determination (R^2) value of 0.998 showed that the equation is highly reliable. A p value less than 0.0001 indicate that the model is statistically significant. The model was found to be adequate for prediction within the range of variable chosen Figs. 5 to 8 shows observed degradation of phenol versus those from the statistical model (Eq. (4)). The figure explains that the predicted data of the response from the empirical model is in good agreement with the experimentally obtained data.

4.1. Effect of glucose

The contour plot represents maximum percentage of phenol degradation against glucose and yeast extract. The maximum percentage of phenol degradation is 98% at a particular range of glucose (0.25–0.38 g/L) and yeast extract (0.15–0.20 g/L), which is also clearly illustrated in Fig. 5. The optimum level of phenol degradation occurs with 98% at glucose (0.34 g/L) and yeast extract (0.165 g/L), calculated by derivatization of the equation (3) and by solving the inverse matrix. Glucose in minimal medium influenced the degradation of phenol. The effect of glucose as shown in Fig. 1 indicates that increase in the concentration of glucose above (0.25–0.38 g/L) showed repressive effect, whereas (0.25 g/L) glucose resulted in only 98% degradation. The synthesis of phenol degrading enzymes and inhibition of phenol utilization by the cells could take place in presence of lower glucose concentration as reported earlier [26–29,37]. However, another strain of *Rhodomonas gracilis* has been reported to utilize phenol in preference to glucose, both in batch and in carbon-limited continuous culture [28]. Phenol metabolism observed in the presence of glucose may be affected at the cell membrane phenol is transported by uptake systems and which is a high inducible affinity system [29].

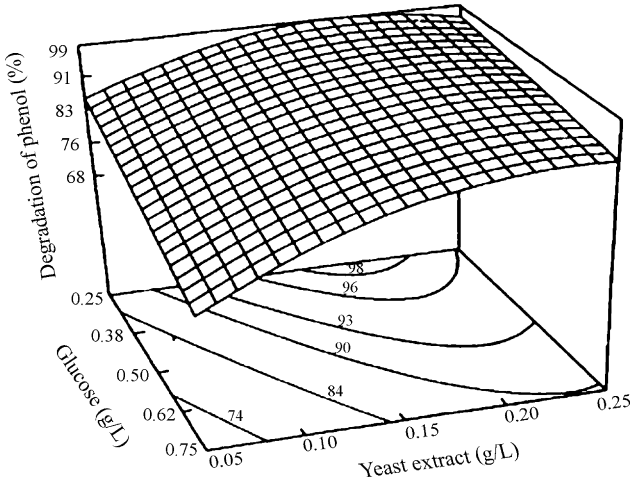

Fig. 5. Phenol degradation on 3-D graphics for response surface optimization versus glucose and yeast extract.

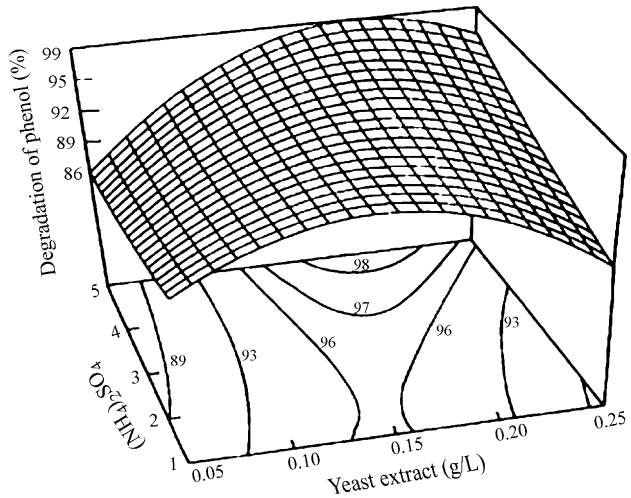

Fig. 6. Phenol degradation on 3-D graphics for response surface optimization versus yeast extract and ammonium sulphate.

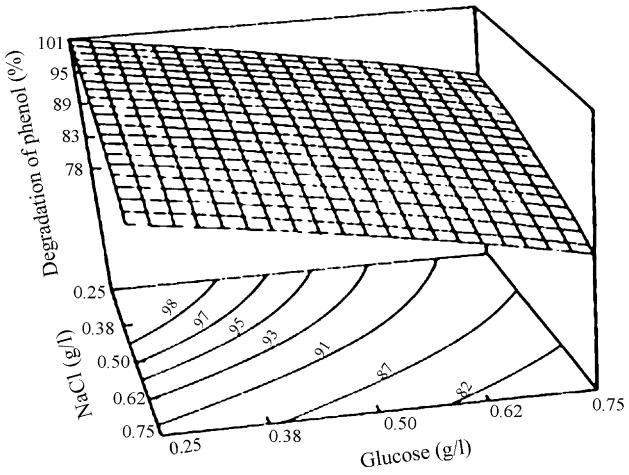

Fig. 8. Phenol degradation on 3-D graphics for response surface optimization versus sodium chloride and glucose.

4.2. Effect of yeast extract

From Fig. 6, the maximum percentage of phenol degradation was found to occur with yeast extract (0.15–2.0 g/L) and $(NH_4)_2SO_4$ (2–3 g/L) at the level of degradation (98%). Optimum level of yeast extract (0.165 g/L) and $(NH_4)_2SO_4$ (2.32 g/L) showed the maximum percentage of phenol degradation as (98%). The concentration of yeast extract in minimal medium (Fig. 2) was varied from 0.05 to 0.25 g/L and there is no considerable increase in the degradation beyond 0.15 g/L. Increasing the concentration of yeast extract from 0.05 to 0.15 g/L increased the degradation from 50 to 98%. But above 0.15 g/L yeast extract, the degradation was decreasing and there is no considerable increase in the degradation beyond 0.15%. Increasing the initial yeast extract concentration not only increases the bacterial yield, but also the time required for completion of the degradation. A number of substances have been reported in the literature that enhances the growth of *R. gracilis* and likely constituents of yeast extract [26]. These include certain amino acids, peptides, and vitamins and several organic acids including pyruvic acid [26–32,37].

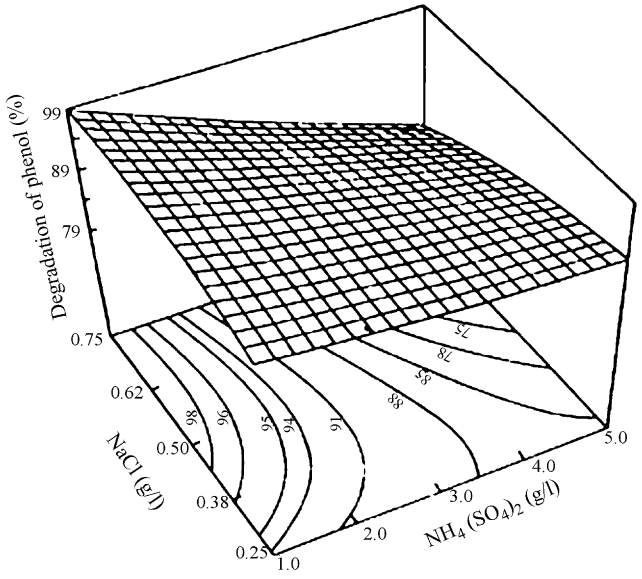

Fig. 7. Phenol degradation on 3-D graphics for response surface optimization versus ammonium sulphate and sodium chloride.

4.3. Effect of $(NH_4)_2SO_4$

Three-dimensional plot representing maximum percentage of phenol degradation (98%) against $(NH_4)_2SO_4$ (2–3 g/L) and NaCl (0.62–0.75 g/L) is shown in Fig. 7. Optimization level of $(NH_4)_2SO_4$ (2.32 g/L) and NaCl (0.476 g/L) were determined at maximum degradation of phenol. Chemical nutrients like nitrogen and sodium chloride play an important role in determining the rates and extent of chemical degradation [29,30,33,37]. To evaluate the influence of various nitrogen sources, ammonium sulphate present in the minimal medium, 98% phenol degradation was achieved by *P. putida* (*ATCC 31800*). It showed that supplementing the minimal medium with yeast extract, ammonium sulphate significantly decreased the toxicity of phenol, as well as increased the cell density. Hence, it was concluded that ammonium sulphate and nitrogen sources in minimal medium supported phenol degradation.

4.4. Effect of sodium chloride

From Fig. 8, the maximum percentage of phenol degradation (98%) with NaCl (0.62–0.75 g/L) and glucose (0.25–0.38 g/L) can be clearly seen. Optimim level of degradation (98%) was at NaCl (0.476 g/L) and glucose (0.34 g/L). Bacterial cells maintain an internal osmotic pressure at about 0.85% solution of NaCl. If the environment has a lower osmotic pressure than the cell (hypotonic), water tends to penetrate into the cell, and higher extracellular osmotic pressure (hypertonic) causes the protoplasm to lose water through the partially permeable cell membrane [8,34–37]. A hypotonic environment is the normal condition for most of the bacteria and they tend to exit in a distended form, maintaining their shape within the cell wall. They

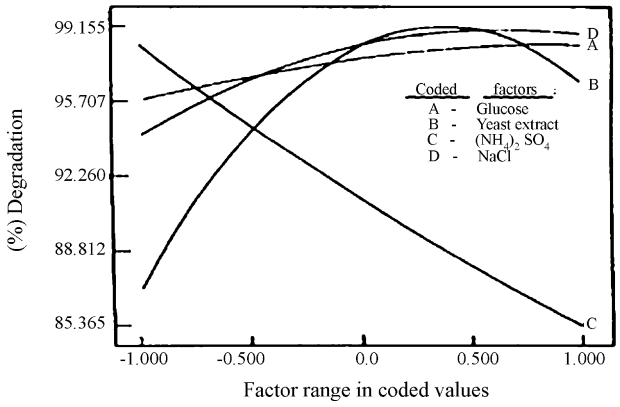

Fig. 9. Factor plot representing the individual variables effect on phenol degradation by *P. putida* (ATCC 31800).

can grow in media with salt concentration varying from less than 0.62 to about 0.75 g/L but their activities are impaired with increasing salinity [31,33].

4.5. Effect of factor plot

The factor effect function of certain factor is a function that describes how the response moves as the level of that factor changes, when the other factors are fixed at their optimum levels. From the trace plot (Fig. 9), it can be observed that each of nutrients used in the present study has its individual effect on biodegradation of phenol by *P. putida* (*ATCC 31800*). The gradual increase in the phenol degradation was seen with increase in glucose from 0.25 g/L (coded value −1) to 0.75 (coded value +1); yeast extracts: 0.05 g/L (coded value −1) to 0.25 g/L (coded value +1) and $(NH_4)_2$ SO_4: 1.0 g/L (coded value −1) to 5 g/L (coded value +1). The optimum level of glucose: 0.75 g/L (coded value 0); yeast extract: 0.15 g/L (coded value 0) and $(NH_4)_2$ SO_4: 1 g/L (coded value 0) were obtained for maximum degradation of phenol. As sodium chloride level is increased, from 0.25 g/L (coded value −1) to 0.75 g/L (coded value +1), the degradation decreased. The optimum level of sodium chloride 0.25 g/L (coded value 0) is obtained for maximum degradation of phenol.

5. Conclusion

The central composite design selected as a response surface method proved to be suitable for performing degradation studies. The true functional relationship between the dependent variable (carbon, nitrogen sources) and maximum percentage of phenol biodegradation have been studied. Response surface methodology was successfully applied to find out the optimum level of the above factors using Box–Behnken design. The optimum conditions for growth and degradation of *P. putida* (*ATCC 31800*) were as follow pH 7.0, temperature 30 °C, glucose (0.34 g/L), yeast extract (0.16 g/L), ammonium sulfate (2.32 g/L) and sodium chloride (0.48 g/L) were investigated. A quadaratic model was obtained for this design expert 7.0. The model employed provided good quality of predictions for the above variables in terms of effective phenol degradation and good correlation coefficient 0.9980 was obtained. By this model, we can predict the response for the above variables at any time. The treatment of phenol in industrial and domestic effluents is very important due to its persistent and toxic effect. The optimum culture medium obtained in these experiments gives a basis for further study with batch (or) fed-batch cultivation in a bioreactor for degradation phenol in dilute industrial effluents.

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