Arab J Sci Eng (2015) 40:2141–2151
DOI 10.1007/s13369-015-1765-z

RESEARCH ARTICLE - CHEMICAL ENGINEERING

Statistical Optimization of Phenol Degradation by *Bacillus pumilus* OS1 Using Plackett–Burman Design and Response Surface Methodology

Sangram S. Patil[1] · **Hara Mohan Jena**[1]

Received: 14 January 2015 / Accepted: 10 June 2015 / Published online: 27 June 2015

Abstract Parameters such as initial phenol concentration, pH, temperature, inoculum size, and concentration of various medium components largely affect the phenol degradation ability of microbes; hence, these parameters must be optimized in order to achieve maximum phenol degradation. The present study is an attempt to optimize phenol degradation by *Bacillus pumilus* OS1, isolated from soil of crude oil spillage site. Experimental design methodology has been adopted for the optimization study. The Plackett–Burman design has determined five significant factors [pH, temperature, phenol concentration, inoculum size, and $(NH_4)_2SO_4$ concentration] out of the nine variables, important for phenol degradation. Response surface analysis using central composite design has been used to study mutual interactions between these variables and to find their optimum levels. The predicted result shows that maximum phenol degradation (99.99 %) could be achieved at pH 7.07, temperature 29.3 °C, phenol 227.4 mg/l, inoculum size 6.3 % (v/v), $(NH_4)_2SO_4$ 392.1 mg/l. The correlation coefficient ($R^2 = 0.9679$) indicates an excellent agreement between the experimental values and predicted ones. A fairly good agreement between the model predicted value and the one obtained from subsequent experimentation at the optimized conditions confirms the validity of the model.

Keywords Phenol · *Bacillus pumilus* OS1 · Crude oil · Plackett–Burman design · Central composite design

✉ Hara Mohan Jena
hara.jena@gmail.com; hmjena@nitrkl.ac.in

[1] Department of Chemical Engineering, National Institute of Technology, Rourkela 769008, Orissa, India

1 Introduction

Phenol and its derivatives are generally found in the effluent of many industries such as paper and pulp mills, steel industries, oil refineries, and several chemical industries processing phenol formaldehyde resin, pesticides, dyes, and pharmaceuticals, etc. [1,2]. Phenol is highly hazardous and xenobiotic, and even at low concentrations, it is lethal to human and aquatic lives. On exposure, phenol exerts a local corrosive effect and mild irritation [3]. Due to these adverse health effects, US Environmental Protection Agency included phenol in the list of priority pollutants and set the limits for its concentration in wastewater discharge as 0.5 mg/l for surface waters and 1 mg/l for the sewerage system [4,5]. World Health Organization (WHO) has also prescribed the limit level of 1 µg/l phenol to regulate its concentration in drinking waters [6]. Therefore, treatment of effluent containing high concentration of phenol is necessary before being discharged into natural bodies. The treatment methods are mainly by physicochemical and biological. Among them, biodegradation offers many advantages as it is environmental friendly and has the ability to completely mineralize phenolic compounds [7]. Microorganisms isolated from a similar type of source but different ecosystem have unlike growth conditions and follow diverse metabolic pathway for the degradation of the substrate. Hence, these microbes exhibit dissimilarity in degradation efficiency and the tolerance potentiality toward the same substrate. Biodegradation of phenol by bacteria has been extensively studied, and large numbers of phenol-degrading bacteria have been isolated and characterized so far [8–11]. *Bacillus pumilus* is one of the potential phenol degraders [12,13]. Therefore, it has broad prospects for the treatment of phenolic effluent.

Phenol degradation is sensitive to many factors such as pH, temperature, inoculum size, carbon sources, and

nitrogen sources [14–16]. Optimization of such nutritional and physical parameters is of primary importance in phenol biodegradation processes as optimum level of factors enhances the degradation efficiency along with significant reduction in the cost of the process. Therefore, it is necessary to design process in order to maximize phenol degradation by *B. pumilus* OS1. There is broad range of modeling and optimization methodologies, which vary from one factor at a time (OFAT) to complex statistical designs such as Plackett–Burman design, central composite design (CCD), and Box–Behnken design (BBD) [17]. The one-factor-at-a-time (OFAT) approach is laborious, time-consuming, and less capable of finding true optimum levels due to the interactions among variables [18]. On the other hand, statistical planned experiments effectively solve such problems and minimize the error in determining the effect of parameters [19]. The design of experiment (DOE) reduces the number of experiments and increases process efficiency [20,21]. Statistically designed experiments are used for optimization strategies such as screening experiments and optimization for targeted response [22]. Statistical experimental designs such as Plackett–Burman and CCD have been successfully used to optimize many bioprocesses [23–26].

The Plackett–Burman design was developed by Plackett and Burman in 1946. It is two-level fractional design for studying up to $k = N - 1$, where k are variables, and N is the number of runs. These designs have complex alias structures, and hence, this design was generally preferred for screening of significant factors [27]. CCD is the most popular type of second-order response surface designs. It is designed to estimate the coefficients of a quadratic model, and it gives reasonable information for testing lack of fit [28]. Optimization of culture conditions for phenol degradation by *B. pumilus* is not available in the literature.

The objective of the present study was to optimize growth conditions and media components for an enhancement of phenol degradation by*B. pumilus* OS1 isolated from soil of a crude oil spillage site. The Plackett–Burman design has been used for screening of significant factors for phenol degradation, and CCD has been employed to find optimum levels of the significant factors.

2 Materials and Methods

2.1 Microorganism and Inoculum Preparation

Bacillus pumilus OS1 has been isolated from the soil collected from crude oil spillage site of Haldia Oil Refinery, Haldia, India. A fresh culture of bacterium grown on nutrient agar slants has been inoculated in nutrient broth and centrifuged at 150 rpm and incubated for 30 °C for 24 h. These cells have been subsequently used as inoculum throughout the study.

2.2 Phenol Degradation

The liquid mineral salt medium (MSM) used initially in the present work has a composition in (mg/l) as: 400 K_2HPO_4, 200 KH_2PO_4, 400 $(NH_4)_2SO_4$, 100 NaCl, 100 $MgSO_4$,10 $MnSO_4{\cdot}H_2O$, 10 $Fe_2(SO_4)_3{\cdot}H_2O$, 10 $Na_2MoO_4{\cdot}2\,H_2O$ [29]. Phenol has been procured from Merck Chemicals (India), and it has been used as sole source of carbon. Initially, the stock solution of 10,000 mg/l phenol has been prepared, and further as per requirement, it has been diluted. Batch mode shake flask experiments have been conducted in 250- ml Erlenmeyer flasks containing 100 ml mineral salt media. All experiments have been performed for 36 h in triplicates, and average of the independent experiments has been taken as the result.

2.3 Phenol Determination

Direct photometric method [30] has been used for the determination of phenol. In this method, phenolic material reacts with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH 7.9 $\pm$ 0.1 to form a stable reddish-brown antipyrene dye with maximum absorbance at 500 nm.

2.4 Experimental Design and Data Analysis

2.4.1 Screening of Significant Factors by Using Plackett–Burman Design

Totally nine variables that are important for phenol biodegradation have been selected for screening via Plackett–Burman design, and each factor has been studied at three levels. The details of variables and their levels are given in Table 1.

Sixteen individual experiments including four center points have been performed for 11 variables (including two dummy variables) to generate regression coefficient values.

The Plackett–Burman design is based on the first-order polynomial model:

$$Y = \beta_0 + \sum \beta_i X_i \tag{1}$$

where Y denotes the response (phenol degradation in %), β_0 is model intercept, β_i is factor linear coefficient, and X_i is the level of the independent factor. From the regression analysis, the factors showing p values below 0.05 have been considered to have significant effect on phenol degradation and further used for optimization by CCD.

2.4.2 Optimization by Response Surface Methodology

The full factorial CCD has been used to study the effect of significant factors on phenol degradation and to find their

Table 1 Eleven variables and their levels used in Plackett–Burman design

Variable code	Variables	Low level (−1)	Central level (0)	High level (+1)
X_1	pH	6	7	8
X_2	Temperature (°C)	25	30	35
X_3	Phenol (mg/l)	150	250	350
X_4	Inoculum size (%, v/v)	3.5	6.5	9.5
X_5	KH_2PO_4 (mg/l)	100	200	300
X_6	K_2HPO_4 (mg/l)	300	400	500
X_7	$(NH_4)_2SO_4$ (mg/l)	300	400	500
X_8	NaCl (mg/l)	50	100	150
X_9	$MgSO_4$ (mg/l)	50	100	150
X_{10}	Dummy 1	−1	0	+1
X_{11}	Dummy 2	−1	0	+1

Table 2 Levels of variables examined in central composite design

Coded level					
Variables	−2	−1	0	+1	+2
pH	6	6.5	7	7.5	8
Temperature (°C)	26	28	30	32	34
Phenol mg/l	150	200	250	300	350
Inoculum size (%, v/v)	3.5	5	6.5	8	9.5
$(NH_4)_2SO_4$ mg/l	300	350	400	450	500

optimum levels. By using Plackett–Burman method, five significant factors for phenol degradation have been obtained. Each of the independent variables has been studied at five different levels $(-\alpha, -1, 0, +1, +\alpha)$, where $\alpha = 2$. The insignificant factors have been kept at their zero level (center value) throughout the study. The details of levels of variables studied are given in Table 2.

A total of 50 experiments have been performed which are having $2^5 = 32$ cube points plus eight center points and 10 axial points. The details of the experimental design are given in Table 5. The second-order polynomial equation has been used to calculate the relationship between the independent variables, and the response is as follows:

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

where Y denotes the response (phenol degradation in %), X_i and X_j are input variables, β_0 is model intercept, β_i is factor estimates, β_{ii} is the i th quadratic coefficient, and β_{ij} is the ij th interaction coefficient. The three-dimensional response surface plots have been constructed for determining interaction among the variables, and further the point prediction method has been used to find the optimum levels of each variable. The statistical software Design-Expert—7.0.0, (Stat-Ease Inc., Minneapolis, USA) (Trial version) has been used for designing experiments, regression analysis, and to plot the response surface graphs.

3 Results and Discussion

3.1 Screening of Significant Factors by Using Plackett–Burman Design

A total of nine variables have been studied with respect to their effect on phenol degradation using Plackett–Burman design. The design of 16 experiments and the corresponding responses are shown in Table 3.

The variation in response suggests that process optimization is important for improving the phenol biodegradation. Analysis of the regression coefficients and the p values of nine variables are shown in Table 4.

Among nine variables, X_1 shows negative effect, while remaining variables (X_2–X_9) show a positive effect on phenol degradation. On the basis of values of coefficient for significance ($p<0.05$), it has been found that among nine variables, pH, temperature, phenol concentration, inoculum size, and $(NH_4)_2SO_4$ concentration have significant effect on phenol degradation by *B. pumilus* OS1. The following model equation has been obtained for phenol degradation (Y):

$$Y = 38.59 - 8.39X_1 + 3.51X_2 + 4.79X_3 + 4.11X_4 + 1.71X_5 + 0.76X_6 + 5.11X_7 + 0.93X_8 + 1.13X_9 \qquad (3)$$

The R^2 (correlation coefficient) value of 0.9752 indicates up to 9y7.52 % variability in phenol degradation could be calculated. The Predicted R^2 of 0.9129 has been in reasonable agreement with the adjusted R^2 of 0.9306.

3.2 Optimization of Significant Factors by Response Surface Methodology

Central composite design estimates interactions between the significant factors and the values of optimal levels. The design matrix of tested variables and the experimental results are represented in Table 5.

Regression equation coefficients have been calculated, and the results of CCD have been fitted with the second-order polynomial model equation. The response, phenol degrada-

Table 3 Plackett–Burman design matrix for nine variables with actual values along with experimentally obtained phenol degradation

pH	Temperature (°C)	Phenol (mg/l)	Inoculum size (%, v/v)	KH_2PO_4 (mg/l)	K_2HPO_4 (mg/l)	$(NH_4)_2SO_4$ (mg/l)	NaCl (mg/l)	$MgSO_4$ (mg/l)	Phenol degradation (%)
8	35	150	9.5	300	500	300	50	50	29.54
6	35	350	3.5	300	500	500	50	50	55.46
8	25	350	9.5	100	500	500	150	50	41.30
6	35	150	9.5	300	300	500	150	150	59.69
6	25	350	3.5	300	500	300	150	150	44.78
6	25	150	9.5	100	500	500	50	150	45.39
8	25	150	3.5	300	300	500	150	50	21.90
8	35	150	3.5	100	500	300	150	150	19.58
8	35	350	3.5	100	300	500	50	150	38.45
6	35	350	9.5	100	300	300	150	50	49.85
8	25	350	9.5	300	300	300	50	150	30.40
6	25	150	3.5	100	300	300	50	50	26.70
7	30	250	6.5	200	400	400	100	100	97.40
7	30	250	6.5	200	400	400	100	100	95.79
7	30	250	6.5	200	400	400	100	100	98.14
7	30	250	6.5	200	400	400	100	100	92.40

Table 4 Effects of the factors and statistical analysis of the Plackett–Burman design

Factors	Effect	Coefficient	F value	p value
Intercept		38.59	21.87	0.0017[a]
X_1–pH	−16.78	−8.39	89.84	0.0002[a]
X_2–Temperature	7.02	3.51	15.70	0.0107[a]
X_3–Phenol	9.57	4.79	29.23	0.0029[a]
X_4- Inoculum size	8.22	4.11	21.53	0.0056[a]
X_5–KH_2PO_4	3.42	1.71	3.72	0.1116
X_6–K_2HPO_4	1.51	0.76	0.73	0.4327
X_7–$(NH_4)_2SO_4$	10.22	5.11	33.33	0.0022[a]
X_8–NaCl	1.86	0.93	1.10	0.3416
X_9–$MgSO_4$	2.26	1.13	1.62	0.2585

$R^2 = 0.9752$; Adj-$R^2 = 0.9306$; Pred-$R^2 = 0.9129$

[a] p value less than 0.05 indicates that model terms are significant

tion (Y) can be expressed in terms of following regression equation:

$$\begin{aligned} Y = {} & 97.14 + 2.68X_1 - 8.32X_2 - 3.84X_3 - 1.80X_4 \\ & -2.06X_5 - 1.92X_1X_2 - 0.085X_1X_3 \\ & +6.57X_1X_4 + 0.36X_1X_5 - 1.72X_2X_3 \\ & +3.12X_2X_4 - 0.44X_2X_5 - 1.45X_3X_4 - 0.68X_3X_5 \\ & +1.70X_4X_5 - 13X_1^2 - 10.32X_2^2 - 3.29X_3^2 \\ & -6.72X_4^2 - 5.98X_5^2 \end{aligned} \quad (4)$$

The above model can be used to predict the percentage degradation of phenol within limits of experimental factors. The R^2 (correlation coefficient) value for regression model has been 0.9679, indicating that experimental results have been best fitted by the quadratic model. Figure 1 shows the experimental response values agree well with predicted response values obtained for phenol degradation by *B. pumilus* OS1.

The adequacy of the model has been checked using analysis of variance (ANOVA) as shown in Table 6. The ANOVA designates the actual relationship between the response, and significant variables denoted by the equation are accurate.

The lack-of-fit F value of 2.26 implies the lack of fit is not significant relative to the pure error. There has been a 13.55 % chance that a lack-of-fit F value this large could occur due to noise. The predicted R^2 of 0.8946 is in reasonable agreement with the R^2 adjusted of 0.9457. Adequate precision measures and the signal-to-noise ratio greater than 4 are desirable, and it has been found that 21.71 indicates an adequate signal. A coefficient of variation is the standard deviation, which has been found as 6.87 %, and its lower value indicates that performed experiments are highly reliable. Three-dimensional response surface plots have been constructed by using Design-Expert software which represents the predicted response over a range of the design surface. Each three-dimensional surface plot describes the effect of two parameters on the response (phenol degradation in %), keeping other factors at their zero levels.

Table 5 Central composite design for five independent variables and their observed and predicted results

Run	pH	Temperature (°C)	Phenol (mg/l)	Inoculum size (% v/v)	$(NH_4)_2SO_4$ (mg/l)	Phenol degradation (%)	
						Observed	Predicted
1	6.5	28	200	5	350	72.80	76.62
2	7.5	28	200	5	350	72.17	72.14
3	6.5	32	200	5	350	68.90	61.92
4	7.5	32	200	5	350	40.04	49.76
5	6.5	28	300	5	350	74.95	76.81
6	7.5	28	300	5	350	72.61	71.99
7	6.5	32	300	5	350	51.14	55.24
8	7.5	32	300	5	350	47.90	42.74
9	6.5	28	200	8	350	59.60	53.16
10	7.5	28	200	8	350	75.34	74.94
11	6.5	32	200	8	350	46.49	50.93
12	7.5	32	200	8	350	68.14	65.02
13	6.5	28	300	8	350	48.11	47.54
14	7.5	28	300	8	350	62.61	68.98
15	6.5	32	300	8	350	36.48	38.43
16	7.5	32	300	8	350	54.62	52.19
17	6.5	28	200	5	450	66.87	70.63
18	7.5	28	200	5	450	69.67	67.57
19	6.5	32	200	5	450	57.20	54.15
20	7.5	32	200	5	450	45.17	43.41
21	6.5	28	300	5	450	67.30	68.09
22	7.5	28	300	5	450	65.82	64.70
23	6.5	32	300	5	450	44.50	44.75
24	7.5	32	300	5	450	25.90	33.67
25	6.5	28	200	8	450	54.10	53.98
26	7.5	28	200	8	450	75.03	77.19
27	6.5	32	200	8	450	46.54	49.97
28	7.5	32	200	8	450	68.96	65.49
29	6.5	28	300	8	450	46.91	45.64
30	7.5	28	300	8	450	68.79	68.50
31	6.5	32	300	8	450	38.53	34.75
32	7.5	32	300	8	450	48.97	49.94
33	6	30	250	6.5	400	39.80	39.79
34	8	30	250	6.5	400	52.65	50.49
35	7	26	250	6.5	400	74.32	72.51
36	7	34	250	6.5	400	39.60	39.25
37	7	30	150	6.5	400	90.50	91.66
38	7	30	350	6.5	400	79.61	76.29
39	7	30	250	3.5	400	78.41	73.87
40	7	30	250	9.5	400	64.31	66.68
41	7	30	250	6.5	300	79.52	77.35
42	7	30	250	6.5	500	69.09	69.10
43	7	30	250	6.5	400	98.56	97.14
44	7	30	250	6.5	400	96.20	97.14
45	7	30	250	6.5	400	98.28	97.14

Table 5 continued

Run	pH	Temperature (°C)	Phenol (mg/l)	Inoculum size (% v/v)	$(NH_4)_2SO_4$ (mg/l)	Phenol degradation (%)	
						Observed	Predicted
46	7	30	250	6.5	400	98.30	97.14
47	7	30	250	6.5	400	98.24	97.14
48	7	30	250	6.5	400	98.18	97.14
49	7	30	250	6.5	400	89.10	97.14
50	7	30	250	6.5	400	98.10	97.14

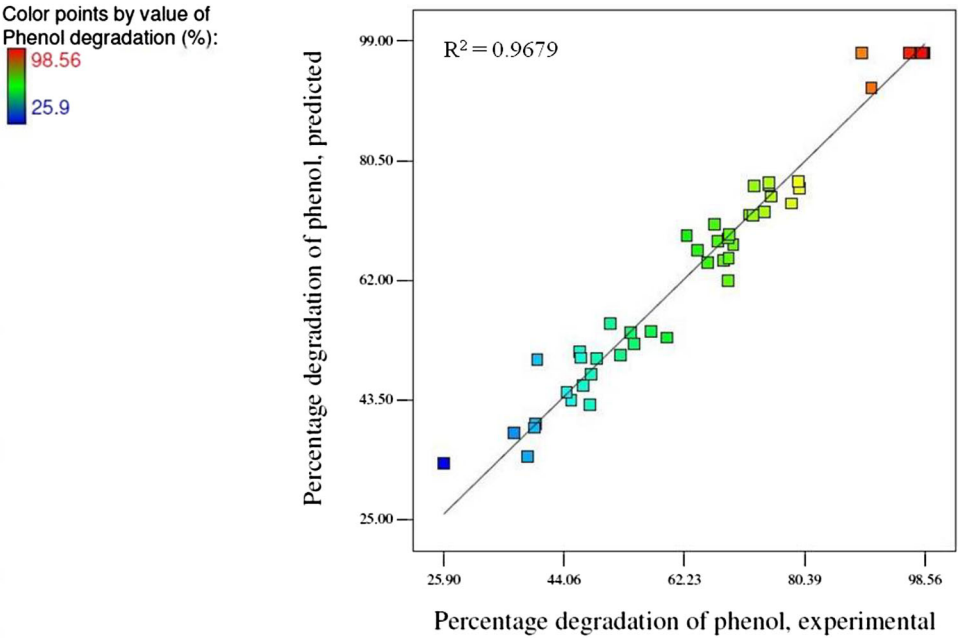

Fig. 1 Predicted versus Experimental percentage of phenol degradation by *Bacillus pumilus* OS1

The effects of the pH (X_1) and temperature (X_2) on the response (Y) at fixed phenol concentration (X_3) of 250 mg/l, inoculum size (X_4) of 6.5 % (v/v), and $(NH_4)_2SO_4$ (X_5) 400 mg/l are shown in Fig. 2a. The response (Y) increases as pH increases from 6 to 7.07, and temperature increases from 26 to 29.2 °C then further Y decreases with increase in pH and temperature. The decrease in response (Y) might be due to change in pH, and temperature affects the solubility and reactivity of enzymatic compounds produced by microbes [14,31]. At these conditions, the model predicts maximum 99.04 % of phenol degradation. This three-dimensional surface plot shows that the effect of an interaction between pH and temperature is significant on response as indicated by low p values (<0.05) (Table 6). The effects of pH (X_1) and phenol concentration (X_3) on the response (Y) while keeping temperature (X_2), inoculum size (X_4), and $(NH_4)_2SO_4$(X_5) at their middle level are shown in Fig. 2b. This three-dimensional surface plot and p value >0.05 (Table 6) suggests that the interaction between pH and phenol concentration is negligible. The effects of pH (X_1) and inoculum size (X_4) on the response (Y) at fixed temperature (X_2), phenol concentration (X_3), and $(NH_4)_2SO_4$(X_5) are shown in Fig. 2c. Response (Y) increases as inoculum size is increased from 3.5 to 6.36 % (v/v) and further decreases with increase in inoculum size. This might be due to the increase in inoculum size reduces the lag phase duration, and beyond optimal value, its effect become marginal [2]. Response (Y) increases as pH is increased from 6 to 7.04 and further decreases with increase in pH. At these conditions, the predicted maximum phenol degradation is 97.33 %. The nature of this three-dimensional surface plot indicates that the effect of the mutual interaction between pH and inoculum size is significant on the response as suggested by low p value (<0.05) (Table 6). The effects of pH (X_1) and $(NH_4)_2SO_4$(X_5) on the response (Y) at fixed temperature (X_2), phenol concentration (X_3), and inoculum size (X_4) are shown in Fig. 2d. The shape of this three-dimensional surface plot and p value indicates that the effect of an interac-

Table 6 Analysis of variance for response surface quadratic model obtained from experimental design

	Sum of squares	*df*	Mean square	*F* Value	*p* value
Model	17 777.23	20	888.86	43.69	<0.0001[a]
X_1–pH	286.33	1	286.33	14.07	0.0008[a]
X_2–Temperature	2766.23	1	2766.23	135.98	<0.0001[a]
X_3–Phenol	590.28	1	590.28	29.02	<0.0001[a]
X_4–Inoculum size	129.31	1	129.31	6.36	0.0174[a]
X_5–$(NH_4)_2SO_4$	170.16	1	170.16	8.36	0.0072[a]
X_1X_2	118.12	1	118.12	5.81	0.0225[a]
X_1X_3	0.23	1	0.23	0.011	0.9158
X_1X_4	1379.18	1	1379.18	67.79	<0.0001[a]
X_1X_5	4.06	1	4.06	0.20	0.6583
X_2X_3	94.26	1	94.26	4.63	0.0398[a]
X_2X_4	310.50	1	310.50	15.26	0.0005[a]
X_2X_5	6.34	1	6.34	0.31	0.5811
X_3X_4	67.51	1	67.51	3.32	0.0788
X_3X_5	14.80	1	14.80	0.73	0.4007
X_4X_5	92.89	1	92.89	4.57	0.0412[a]
X_1^2	5407.58	1	5407.58	265.81	<0.0001[a]
X_2^2	3405.27	1	3405.27	167.39	<0.0001[a]
X_3^2	346.79	1	346.79	17.05	0.0003[a]
X_4^2	1443.24	1	1443.24	70.94	<0.0001[a]
X_5^2	1144.14	1	1144.14	56.24	<0.0001[a]
Residual	589.96	29	20.34		
Lack of fit	517.15	22	23.51	2.26	0.1355
Pure error	72.82	7	10.40		
Cor total	18 367.19	49			

$R^2 = 0.9679$; Adj-$R^2 = 0.9457$; Pred-$R^2 = 0.8946$; C.V. (Coefficient of variation) = 6.87 %

[a] p value less than 0.05 indicate model terms are significant

tion between pH and $(NH_4)_2SO_4$ is insignificant on response (Y).

Temperature (X_2) and phenol concentration (X_3) effects on the response (Y), keeping pH (X_1), inoculum size (X_4), and $(NH_4)_2SO_4$ (X_5) at zero levels are shown in Fig. 2a. The response (Y) increases as concentration of phenol is increased from 150 to 225.6 mg/l and further rapidly decreases with increase in phenol concentration. The quick decrease in response might be due to inhibition effect phenol as a substrate [32]. The response (Y) increases as the temperature is increased from 28 to 29.3 °C and further decreases with increase in temperature. At these conditions, the maximum 97.33 % phenol degradation is predicted by the model. The mutual interaction between temperature and phenol has a significant effect on response and is indicated by three-dimensional plot (Fig. 3a) and low p value (<0.05) (Table 6). The effects of temperature (X_2) and inoculum size (X_4) on the response (Y) at fixed pH (X_1), phenol concentration (X_3), and $(NH_4)_2SO_4$(X_5) are shown in Fig. 3b. The nature of this three-dimensional surface plot suggests that the effect of a mutual interaction between temperature and inoculum size is significant on response. Temperature (X_2) and $(NH_4)_2SO_4$ (X_5) effects on the response (Y) while keeping pH (X_1), phenol concentration (X_3), and inoculum size (X_4) at zero levels are shown in Fig. 3c. From this three-dimensional surface plot and p value >0.05 (Table 6), it is found that the effect of an interaction between temperature and $(NH_4)_2SO_4$ is insignificant on response. The effects of phenol concentration (X_3) and inoculum size (X_4) on the response (Y) at fixed pH (X_1), temperature (X_2), and $(NH_4)_2SO_4$ (X_5) are shown in Fig. 3d. The shape of this three-dimensional surface plot and p value suggests that the interaction between phenol and inoculum size is insignificant on response.

The effects of phenol concentration (X_3) and $(NH_4)_2SO_4$(X_5) on the response (Y) at fixed pH (X_1), temperature (X_2), and inoculum size (X_4) are shown in Fig. 4a. From the nature of this three-dimensional surface plot and p value, it is found that the interaction between phenol and $(NH_4)_2SO_4$ concentration is insignificant on

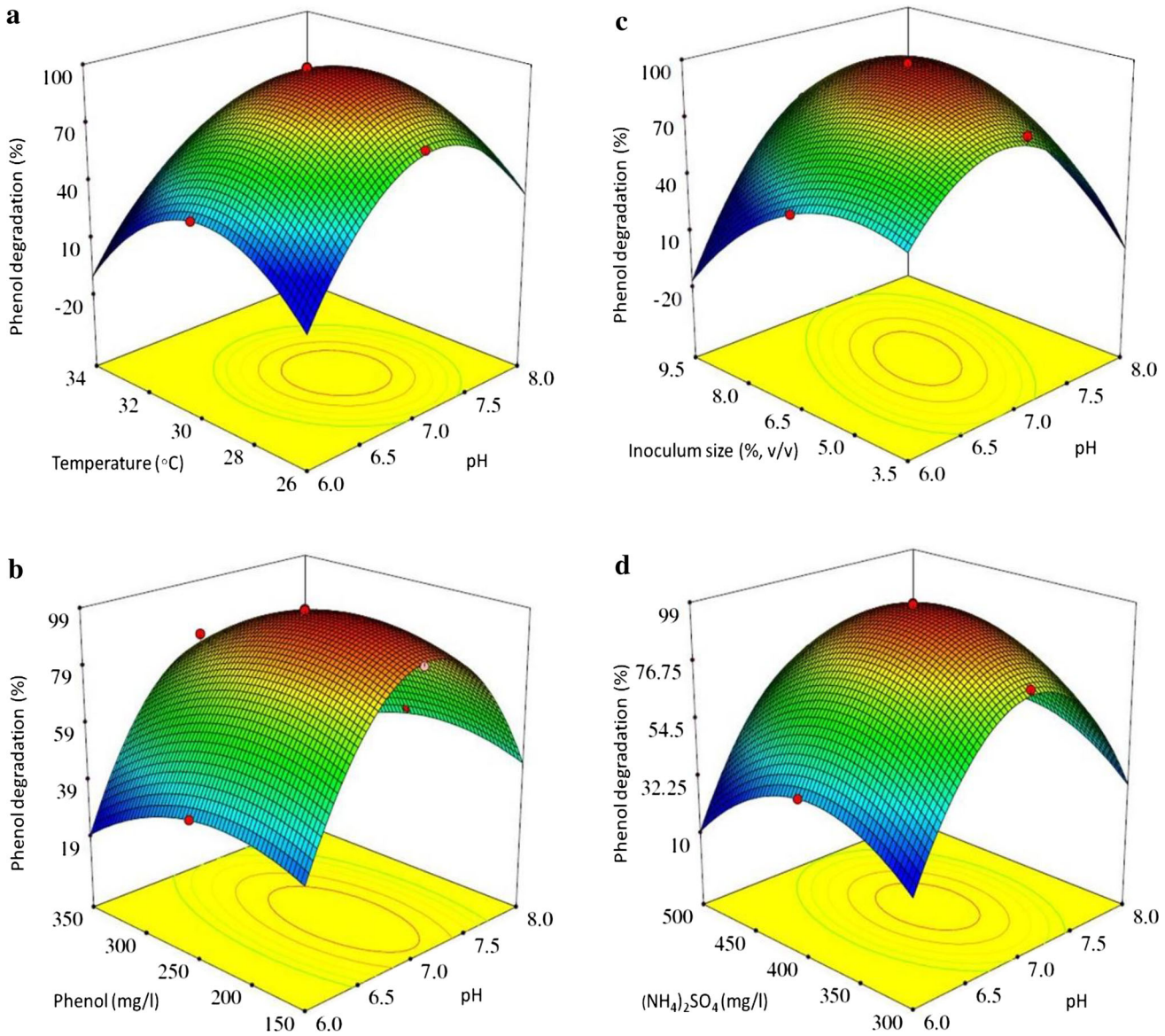

Fig. 2 Three-dimensional response surface plots of the effect of variable interactions on phenol degradation by *Bacillus pumilus* OS1 **a** pH and temperature; **b** pH and phenol; **c** pH and inoculum size; **d** pH and $(NH_4)_2SO_4$

response. Inoculum size (X_4) and $(NH_4)_2SO_4$(X_5) effects on the response (Y) while keeping pH (X_1), temperature (X_2), and phenol concentration (X_3) at zero levels are shown in Fig. 4b. The response (Y) increases as $(NH_4)_2SO_4$ concentration is increased from 300 to 390.3 mg/l and further response (Y) decreases with increase in $(NH_4)_2SO_4$ concentration. This has been likely because the addition of $(NH_4)_2SO_4$ in media significantly decreases the toxicity of phenol and hence enhances cell growth [16]. This three-dimensional surface plot and low p value (<0.05) (Table 6) indicates that the effect of an interaction between inoculum size and $(NH_4)_2SO_4$ is significant on response.

The point prediction feature of CCD has been used to determine optimum levels of each variable for maximum phenol degradation (%), and these are as follows: pH 7.07, temperature 29.3 °C, phenol 227.4 mg/l, inoculum size 6.3 % (v/v) and $(NH_4)_2SO_4$ 392.1 mg/l. Under these optimized conditions, predicted phenol degradation is 99.99 %.

3.3 Validation of Experimental Designs

Validation of obtained statistical model has been done by performing phenol degradation experiment at pH 7.07, tem-

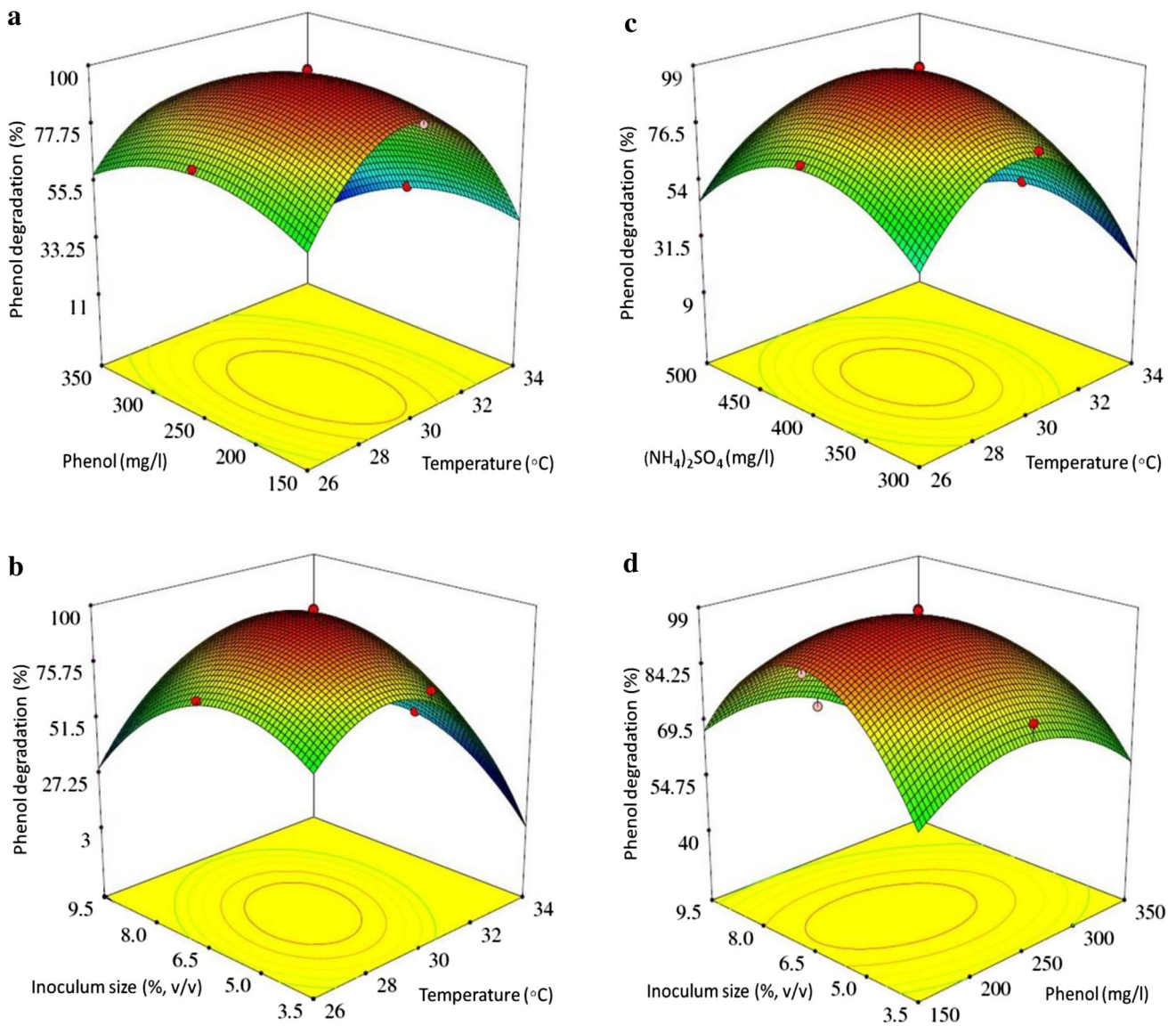

Fig. 3 Three-dimensional response surface plots of the effect of variable interactions on phenol degradation by *Bacillus pumilus* OS1 **a** temperature and phenol; **b** temperature and inoculum size; **c** temperature and $(NH_4)_2SO_4$; and **d** phenol and inoculum size

perature 29.3 °C, phenol 227.4 mg/l, inoculum size 6.3 % (v/v), and $(NH_4)_2SO_4$ 392.1 mg/l. At these optimized conditions, the predicted response for phenol degradation is 99.99 %, and the average of experimental values is 99.87 %. The experimental value is close to the experimental value and represents the validity of the model.

4 Conclusions

To identify significant factors by screening important variables for phenol degradation by *B. pumilus* OS1 isolated from crude oil spillage site and to determine the interaction between them, statistical design of the experiments has been carried out. Nine variables have been tested using Plackett–Burman design, which resulted in five significant factors (pH, temperature, phenol concentration, inoculum size, and $(NH_4)_2SO_4$ concentration). CCD has been applied for optimization of these factors. A quadratic model has been developed which accurately predicts the levels of variables for maximum phenol degradation as: pH 7.07, temperature 29.3 °C, phenol 227.4 mg/l, inoculum size 6.3 % (v/v), and $(NH_4)_2SO_4$ 392.1 mg/l. The model has been validated by further experimentation, and the predicted response (99.99 %) has been found to be very much close to the experimental value of 99.87 % at the optimized conditions. The model can

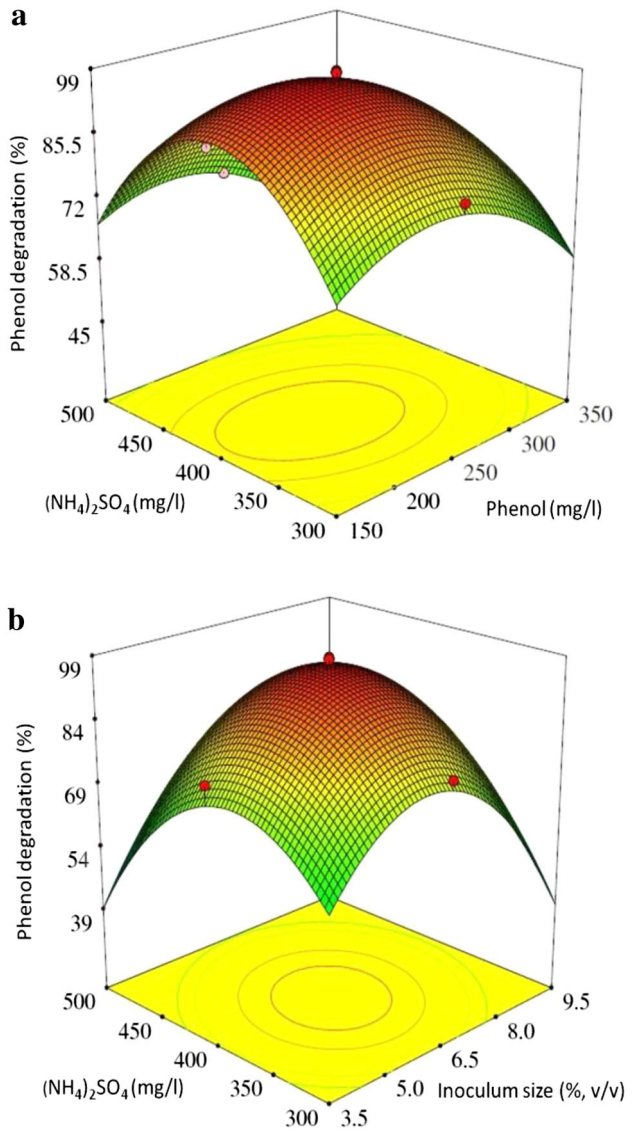

Fig. 4 Three-dimensional response surface plots of the effect of variable interactions on phenol degradation by *Bacillus pumilus* OS1 **a** phenol and $(NH_4)_2SO_4$; **b** inoculum size and $(NH_4)_2SO_4$

be suitably used for the prediction phenol biodegradation and design of bioreactors carrying phenol biodegradation with *B. pumilus* OS1.

References

1. Carron, JM.; Afghan, B.K. : Environmental aspects and analysis of phenols in the aquatic environment. In: Afghan, B.K.; Chau, A.S.Y. (eds.) Analysis of trace organics in the aquatic environment, pp. 119–150. CRC Press, Florida (1989)
2. Arutchelvan, V.; Kanakasabai, V.; Elangovan, R.; Nagarjan, S.; Muralikrishnan, V.: Kinetics of high strength phenol degradation using *Bacillus brevis*. J. Hazard. Mater. **B129**, 216–222 (2006)
3. Agency for Toxic Substances and Disease Registry (ATSDR): Toxicological profile for phenol. US Department of Health and Human Services, Georgia (2008)
4. US EPA (Environmental Protection Agency): Phenol ambient water quality criteria. Office of Planning and Standards, Washington D.C., USA (1979)
5. Shailubhai, K.: Treatment of petroleum industry oil sludge in soil. Trends Biotechnol. **4**(8), 202–206 (1986)
6. Sa, C.S.A.; Boaventura, R.A.R.: Biodegradation of phenol by *Pseudomonas putida* DSM 548 in a trickling bed reactor. Biochem. Eng. J. **9**, 211–219 (2001)
7. Prpich, G.P.; Daugulis, A.J.: Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two phase partitioning bioreactor. Biodegradation **16**, 329–339 (2005)
8. Ali, S.; Fernandez-Lafuente, R.; Cowan, D.A.: Meta-pathway degradation of phenolics by thermophilic *Bacilli*. Enzyme Microb. Technol. **23**, 462–468 (1998)
9. Alva, V.A.; Peyton, B.M.: Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: influence of pH and salinity. Environ. Sci. Technol. **37**, 4397–4402 (2003)
10. Yamaga, F.; Washio, K.; Moikawa, M.: Sustainable biodegradation of phenol by *Acinetobacter calcoaceticus* P23 isolated from the Rhizosphere of duckweed *Lemna aoukikusa*. Environ. Sci. Technol. **44**, 6470–6474 (2010)
11. Safont, B.; Vitas, A.I.; Penas, F.J.: Isolation and characterization of phenol degrading bacteria immobilized onto cyclodextrin-hydrogel particles within a draft tube spouted bed bioreactor. Biochem. Eng. J. **64**, 69–75 (2012)
12. Gunther, K.; Schlosser, D.; Fritsche, W.: Phenol and cresol metabolism in *Bacillus pumilus* isolated from contaminated groundwater. J. Basic Microbiol. **35**(2), 83–92 (1995)
13. Gayathri, K.V.; Vasudevan, N.: Enrichment of phenol degrading moderately halophilic bacterial consortium from saline environment. J. Biorem. Biodegrad. **1**(1), 104 (2010)
14. Bandyopadhyay, K.; Das, D.; Maiti, B.R.: Kinetics of phenol degradation using *Pseudomonas putida* MTCC 1194. Bioprocess Eng. **18**, 373–377 (1998)
15. Balamurugan, P.; Preetha, B.; Virithagiri, T.: Study on effect of operating parameters on biodegradation of phenol by *Aspergillus Fumigatus*. Int. J. Eng. Res. Appl. **2**((2), 981–986 (2012)
16. Annadurai, G.; Ling, L.Y.; Lee, J.: Statistical optimization of medium components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*. J. Hazard. Mater. **151**, 171–178 (2008)
17. Singh, Y.; Srivastava, S.K.: Statistical and evolutionary optimization for enhanced production of an anti-leukemic enzyme, L-asparaginase, in a protease-deficient *Bacillus aryabhattai* ITBHU02 isolated from the soil contaminated with hospital waste. Indian J. Exp. Biol. **51**, 322–335 (2013)
18. Tang, X.; He, G.; Chen, Q.; Zhang, X.; Ali, M.A.M.: Medium optimization for the production of thermal stable β-glucanase by *Bacillus subtilis* ZJF-1A5 using response surface methodology. Bioresour. Technol. **93**, 175–181 (2004)
19. Lakshmi, M.V.V.C.; Sridevi, V.; Rao, M.N.; Swamy, A.V.N.: Optimization of phenol degradation from *Pseudomonas aeruginosa* (NCIM 2074) using response surface methodology. Int. J. Res. Pharm. Chem. **1**(4), 925–935 (2011)
20. Senthilkumar, S.; Perumalsamy, M.; Prabhu, H.J.; Basha, C.A.; Anantharaman, N.: Response surface optimization for efficient dye removal by isolated strain *Pseudomonas* sp. Cent. Eur. J. Eng. **2**(3), 425–434 (2012)
21. Chen, X.; Bai, J.; Cao, J.; Li, Z.; Xiong, J.; Zhang, L.; Hong, Y.; Ying, H.: Medium optimization for the production of cyclic adenosine 30,50-monophosphate by *Microbacterium* sp. no. 205 using response surface methodology. Bioresour. Technol. **100**, 919–924 (2009)

22. Abdel-Fattah, Y.R.; Saeed, H.M.; Gohar, Y.M.; El-Baz, M.A.: Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs. Process Biochem. **40**, 1707–1714 (2005)
23. Gaur, R.; Gupta, A.; Khare, S.K.: Lipase from solvent tolerant *Pseudomonas aeruginosa* strain: Production optimization by response surface methodology and application. Bioresour. Technol. **99**, 4796–4802 (2008)
24. Reddy, L.V.A.; Wee, Y.; Yun, J.; Ryu, H.: Optimization of alkaline protease production by batch culture of *Bacillus* sp. RKY3 through Plackett–Burman and response surface methodological approaches. Bioresour. Technol. **99**, 2242–2249 (2008)
25. Zhou, J.; Yu, X.; Ding, C.; Zhiping, W.; Zhou, Q.; Pao, H.; Cai, W.: Optimization of phenol degradation by *Candida tropicalis* Z-04 using Plackett–Burman design and response surface methodology. J. Environ. Sci. **23**(1), 22–30 (2011)
26. Suhaila, Y.N.; Ramanan, R.N.; Rosfarizan, M.; Latif, I.A.; Ariff, A.B.: Optimization of parameters for improvement of phenol degradation by *Rhodococcus* UKMP-5M using response surface methodology. Ann. Micobiol. **63**, 513–521 (2013)
27. Myers, R.H.; Montgomery, D.C.; Anderson-cook, C.M.: Response surface methodology: process and product optimization using designed experiments. 3rd edn. Wiley, New Jersey (2009)
28. Breyfogle, F.W.: Statistical methods for testing, development and manufacturing. Wiley, New York (1992)
29. Bai, J.; Wen, J.; Li, H.; Jiang, Y.: Kinetic modelling of growth and biodegradation of phenol and m-cresol using *Alcaligenes faecalis*. Process Biochem. **42**, 510–517 (2007)
30. Clesceri, L.S.; Greenberg, A.E.; Eaton, A.D.: Standard Methods for the Examination of Water and Wastewater, 20th edn. APHA American Public Health Association, Washington D.C., USA (1998)
31. Banerjee, A.; Ghoshal, A.K.: Isolation and characterization of hyper phenol tolerant *Bacillus* sp. from oil refinery and exploration sites. J. Hazard. Mater. **176**, 85–91 (2010)
32. Scragg, A.: The effect of phenol on the growth of *Chlorella vulgaris* and *Chlorella* VT-1. Enzyme Microb. Technol. **39**, 796–799 (2006)