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Biodegradation of 2,4-dichlorophenol by *Bacillus endophyticus* strain: optimization of experimental parameters using response surface methodology and kinetic study

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ABSTRACT

In the present study, the optimization of various experimental parameters, i.e. pH, temperature (°C), inoculum size % (v/v) and ammonium sulphate concentration (g L^{-1}) at different levels were carried out to enhance the 2,4-dichlorophenol (2,4-DCP) degradation by newly isolated *Bacillus endophyticus* strain CP1R. The central composite design of response surface methodology (RSM) was applied for the optimization process. It was observed that the maximum biodegradation efficiency of 100% was achieved for 50 mg L^{-1} 2,4-DCP at optimized condition, i.e. pH 7.36, temperature 35.1°C, inoculum size 10 % (v/v) and $(NH_4)_2SO_4$ concentration 1.4 g L^{-1}, which was up to 27% higher than that obtained at unoptimized condition. Also the inhibition effect of 2,4-DCP was found to be diminished and degradation capacity of the strain increases significantly. The strain was able to degrade 2,4-DCP efficiently up to 400 mg L^{-1} with negligible inhibition effect. The biodegradation kinetic of 2,4-DCP at different initial concentration was carried out and the bio-kinetic parameters obtained was R_m = 1.53 mg DCP h^{-1} L^{-1} and K_s = 711.5 mg L^{-1}.

Keywords: 2,4-dichlorophenol; *Bacillus endophyticus*; Optimization; Biodegradation; Kinetic; Central composite design

1. Introduction

Chlorophenol compounds have toxic and hazardous effect on environment due to its widespread occurrence and industrial production. Chlorophenol compounds have been listed as priority pollutants by the US Environmental Protection Agency [1]. The primary sources of chlorophenols are agricultural application, pulp and paper, lather tanning, biocide, herbicide, chlorination of drinking and wastewater [2–4]. Chlorophenol compounds have been reported for their mutagenicity, carcinogenicity, immunogenicity and fatality [5,6]. The different treatment technologies including physicochemical such as adsorption, photodegradation, chemical oxidation and biological treatment have been developed for the treatment of chlorophenol contamination. However, the physicochemical treatments have disadvantage of high cost, low energy efficient and production of toxic by-product [3,7]. The biological treatment, i.e. use of microorganism and enzyme is potential alternative with promising removal efficiency and eco friendly [2,8,9].

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Bacillus endophyticus strain CP1R was previously isolated by author from the sludge collected from the dye industries effluent treatment plant. The isolated strain has been able to tolerate 2,4-DCP up to 300 mg L^{-1}. However, the surrounding environment has significant effect on the micro-organism growth and the optimization of culture condition can significantly improve the degradation efficiency. Chlorophenols are toxic and has inhibitory effect on growth of micro-organism. This inhibitory effect of micro-organism can be diminished to some extent by optimizing the experimental parameters. Different experimental parameters such as pH, temperature, nutrient, substrate concentration, etc. affects the degradation capability of 2,4-DCP by altering the growth of micro-organism and physical properties of the compound, i.e. 2,4-DCP [10,11]. Each micro-organism has specific range of pH for maximum growth, extreme pH value, i.e. acidic or alkaline condition is inhibitory to the bacteria. During the 2,4-DCP the value of pH drops which affects the growth and degradation capacity of bacteria [12,13]. Temperature affects the growth and activity of micro-organism. At higher temperature, enzymes lose their structure and reduce the degradation efficiency. Also at the low temperature, bacterial activity decreases which increases the inhibitory effects of toxic compounds. The optimum temperature range used for biodegradation by micro-organism is 25 to 35˚C [12,14]. The addition of different carbon and nitrogen source such as glucose, $(NH_4)_2SO_4$, peptone, etc. also affects the degradation capacity of the micro-organism [11,15,16]. Hence, optimization of experimental conditions such as pH, temperature, inoculum size and $(NH_4)_2SO_4$ can improve the degradation efficiency of the *B. endophyticus* strain CP1R. Optimization with conventional method, i.e. single parameter at once is time consuming and less economical. However, optimization by using statistical method such as response surface methodology (RSM) is more suitable as it simultaneously optimize the various parameters and their interaction effects at different levels.

RSM is an important statistical and mathematical design used for determining the influence of different factors on desired response and optimizing the desired response [17]. The RSM is more economical and time saving than conventional techniques as the minimum numbers of experiments were required for getting the desired optimum response. Several studies have been reported that used the RSM for optimization process. In one such study, Box–Behnken design of experiment was used for evaluating the effect of environmental parameters, i.e pH, temperature, time and enzyme concentration on biodegradation of 2,4-DCP with laccase from *Pleurotus* sp. The maximum degradation of 2,4-DCP, approximately 98% was obtained at pH 6, temperature of 40˚C, time 9 h and an enzyme concentration of 8 IU mL^{-1} [18]. In another study, the central composite design of RSM was used for optimizing the multiple responses, i.e. maximum 4-CP biodegradation and specific growth rate [13]. The 4-CP biodegradation efficiency was found 23% higher at RSM optimized condition than that obtained at unoptimized culture conditions. The Box–Behnken design of experiment was used for optimization of the enzymatic conversion of widely available lignocellulosic biomass-wheat straw (WS) and the combined effects of enzyme loading, substrate concentration, surfactant concentration and reaction time on hydrolysis yield from enzymatic saccharification of WS were successfully studied [19].

In the present study, the biodegradation of 2,4-DCP was carried out by *B. endophyticus* strain CP1R. The different experimental parameters were optimized to achieve the maximum biodegradation of 2,4-DCP and biodegradation kinetic parameters were calculated for the same at optimized conditions. This is the first study showing the biodegradation of 2,4-DCP by *B. endophyticus* strain and its optimization to best of our knowledge. The data obtained will be beneficial for effective removal of 2,4-DCP and other chlorophenol derivatives from the environment by the isolated strain.

2. Material and methods

2.1. Chemicals and reagents

Analytical grade 2,4-DCP (purity 98%) was supplied by Loba chemie, India. The stock solution of 2,4-DCP is prepared in 0.02 M NaOH and pH was adjusted to 7.4 ± 0.2 by 1 M ortho-phosphoric acid. All other inorganic chemicals used in the experiments were of analytical grade and obtained from Merck, India. HPLC grade reagents were obtained from Hi-media, India for HPLC analysis.

2.2. Micro-organism

The micro-organism used in this study was previously isolated from sludge collected from the dye industries effluent treatment plant, Gujarat, India. The isolate was identified as *B. endophyticus* strain CP1R (GenBank Accession Number: KM259919) by 16s rDNA sequence-based molecular technique. This isolated *Bacillus* strain was able to resist and degrade 2,4-DCP up to 300 mg L^{-1} at 30˚C and pH 7 based on previous studies. The organism was maintained on

MSM agar slant with 1 g L^{-1} peptone, 50 mg L^{-1} 2,4-DCP and 1.5% agar, pH 7.0 ± 0.1.

2.3. 2,4-DCP degradation study

All the experiment of biodegradation of 2,4-DCP was performed in 250 mL Erlenmeyer with 50-mL mineral salt medium (modified DSMZ-465) having composition of (g L^{-1}): $Na_2HPO_4{\cdot}2H_2O$ 3.5, KH_2PO_4 1, $(NH_4)_2SO_4$ 0.2, $MgCl_2{\cdot}6H_2O$ 0.1, $NaNO_3$ 0.05 and 1 mL of trace element solution having composition of (g L^{-1}): EDTA 0.5, $FeSO_4{\cdot}7H_2O$ 0.2, $CuCl_2{\cdot}2H_2O$ 0.001, $ZnSO_4{\cdot}7H_2O$ 0.01, $MnCl_2{\cdot}4H_2O$ 0.003, $CoCl_2{\cdot}6H_2O$ 0.02, H_3BO_3 0.03, $Na_2MoO_4{\cdot}2H_2O$ 0.003. The 2,4-DCP (50 mg L^{-1}) was added to the medium after autoclaving by filter sterilizing using 0.22 μm syringe filter. The inoculum was prepared in MSM with 50 mg L^{-1} 2,4-DCP and 1 g L^{-1} peptone and incubated for 36 h in rotary shaker.

2.4. Response surface methodology

Optimization of experimental factors to achieve maximum biodegradation of 2,4-DCP by *B. endophyticus* strain CP1R was performed using RSM. The experimental factors selected for optimization were pH, temperature (°C), inoculum size % (v/v) and $(NH4)_2SO_4$ concentration (g L^{-1}). The central composite design was used for the optimization of experimental factors to achieve maximum biodegradation of 2,4-DCP. The total number of experimental runs were; $n = 2^k + 2k + n_0 = 31$, where ($k = 4$) is the independent variables and ($n_0 = 7$) is the number of centre points used in the design. The default value of $\alpha = 2$ was taken in the experiment. The factor levels are coded as −1 (low), 0 (central point) and +1 (high). Table 1 represents the coded and actual values of the factor levels used in the experiments. The relationship between the coded and actual value is described by following equation:

$$X_i = \frac{U_i - U_0}{\Delta U} \quad (1)$$

where X_i is the coded level of the independent variable, U_i is the actual level of the independent variable, U_0 is the uncoded level of the independent variable at its centre point and ΔU is the step change value.

The second-order polynomial regression model used for fitting the experimental data by response surface method is defined as:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_i \sum_j \beta_{ij} X_i X_j \quad (2)$$

where Y = predicted response, k = number factor variables, X_i and X_j = independent variables. The second order regression model is significant in improving the optimization process as it count the interaction effect between variables and surface curvature. The adequacy of the fitted regression model is checked using the analysis of variance (ANOVA) and regression analysis. The robustness of the model can be checked with the help of coefficient of determination (R^2-value) and p-value. All the statistical analysis was performed using the Minitab v16 (trial version), USA.

2.5. Biodegradation kinetic of 2,4-DCP

The kinetic study of biodegradation of 2,4-DCP was performed in 250 mL Erlenmeyer flask containing 50 mL MSM at optimized condition with different initial DCP concentration of 25, 50, 100, 200, 300 and 400 mg L^{-1}. The control flask containing 50 mg L^{-1} 2,4-DCP without inoculum was set to check abiotic loss of DCP. The initial 10 % (v/v) inoculum was added to the medium which has an average biomass concentration of 55 mg dry weight. Culture samples were taken at regular interval to analyse residual 2,4-DCP, chloride ion and biomass concentration. All the experiments were done in duplicate and the mean value of the result is shown.

Table 1
Independent variables and their corresponding levels used in the optimization study

Factors	Coded unit	$-\alpha$	−1	0	+1	$+\alpha$
pH	X_1	5	6	7	8	9
Temperature (°C)	X_2	20	25	30	35	40
Inoculum size[a] % (v/v)	X_3	2	4	6	8	10
$(NH_4)_2SO_4$ (g L^{-1})	X_4	0	0.5	1	1.5	2

[a]1 mL inoculum equals to ~11 mg dry biomass.

2.6. Analytical methods

Biomass concentration is determined by measuring optical density at 600 nm by UV–visible spectrophotometer (Shimadzu UV-1800, Japan). The residual concentration of 2,4-DCP is determined by HPLC system (Jasco, US) coupled with MD-2015 photodiode array detector and 2080 plus isocratic pump. The 1 mL sample is centrifuged at 10,000 rpm for 12 min and supernatant is filtered through 0.22 μm filter before analysis. The sample volume taken was 20 μL. The column used was Ascentis C18 (15 mm × 2.1 mm); sample is eluted at flow rate of 0.75 mL min^{-1} with mobile phase consist of methanol: water: acetic acid (60:38:2%); detection wavelength is 284 nm where maximum absorbance has occurred.

The concentration of chloride ions in the samples was analysed colorimetrically by EPA 9215 method [20]. 0.5-mL culture supernatant is mixed with 0.5-mL colour reagent and mixture is kept for 10 min for colour development. Colour reagent is prepared by equally mixing solution A (saturated solution of mercuric thiocyanate in methanol) and solution B (20.2% ferric nitrate solution in 9 M nitric acid). The absorbance was measured by UV–vis spectrophotometer (Shimadzu UV-1800, Japan) at 460 nm. The chloride ion as quantified against the standard curve of sodium chloride (5–200 mg L^{-1}) [20,21].

3. Results and discussion

3.1. Optimization of experimental factors using RSM

The optimization of experimental factors for maximizing the 2,4-DCP biodegradation by *B. endophyticus* strain CP1R was successfully performed as per central composite design. The effect of all four independent variables i.e. pH, temperature (°C), inoculum size % (v/v) and $(NH_4)_2SO_4$ (g L^{-1}) at different levels was analysed on 2,4-DCP biodegradation. The experimental condition and corresponding per cent degradation obtained was summarized in Table 2. The experimental data were analysed in terms of second-order polynomial equation.

The value for coefficient of determination (R^2) and adjusted R^2 for regression model is 0.963 and 0.932 which are close to 1 (Fig. 1), which explains that the model sufficiently fits the data. The *F*-value and *p*-value was used to test the significance of the regression model. Higher the *F*-value means that the model significantly explains the relation between the dependent and independent variables. The observed *F*-value for the regression model is 30.38 which is greater than critical *F*-value ($F_{0.05,14,16} = 2.33$) at significant level of $p = 0.05$. This implies that the regression model is significant and sufficiently explains all the variation. The critical $F_{0.05,4,16} = 3$ which is less than the calculated *F*-value implying that the linear and square terms of the regression model for 2,4-DCP degradation are significant. The $F_{0.05,6,16} = 2.74$ which is slightly lower than calculated $F = 6.38$ implying the little less significance of the interaction terms of the regression model.

The ANOVA and regression coefficient for 2,4-DCP degradation was summarised in Tables 3 and 4, respectively. From the ANOVA table it was conclusive that there was a significant interaction between the factors. The small *p*-values of interaction and square terms indicate that there is a curvature in the regression surface. Also the small *p*-values for the linear and square terms indicate their significant contribution to the model.

From the Table 4, it was concluded that the main effect of temperature and inoculum size was significant at an individual significant level of 0.05. The small *p*-value ($p < 0.05$) for quadratic terms pH × pH, Temperature × Temperature and $(NH_4)_2SO_4 \times (NH_4)_2SO_4$ concentration and also for the interaction terms $(NH_4)_2SO_4 \times (NH_4)_2SO_4$, pH × Temperature, Temperature × Inoculum size, Temperature × $(NH_4)_2SO_4$ indicate their significance in the regression model. Thus, the regression model equation (in uncoded form) showing the effect of all four independent variable including interaction effect on 2,4-DCP biodegradation can be presented as below:

$$Y = -379.97 + 9.17X_2 - 6.23X_3 - 8.26X_1^2 - 0.3X_2^2 - 11.56X_4^2 + 1.16X_1X_2 + 0.29X_2X_3 + 1.37X_2X_4 \quad (3)$$

where Y = 2,4-DCP biodegradation, X_1 is pH, X_2 is temperature (°C), X_3 is inoculum size % (v/v) and X_4 is $(NH_4)_2SO_4$ (g L^{-1}).

The interaction effect of two independent variables, while keeping other variables at middle point setting, on response was well illustrated using the contour plots. The interaction effect of inoculum size and temperature was illustrated in Fig. 2(a). From the contour plot it was seen that the per cent degradation increases with the increase in inoculum size and temperature. The response increases linearly with inoculum size whereas it was at maximum for temperature in the range 35–40°C. The contour plot between temperature and pH was elliptical showing interaction effect on the response as shown in Fig. 2(b). The per cent degradation was at maximum for the temperature around 35°C and pH around 7.2–7.5.

Table 2
Central composite design of experiments and % degradation of 2,4-DCP

Run order	pH	Temperature (°C)	Inoculum size % (v/v)	$(NH_4)_2SO_4$ (g L^{-1})	% Degradation	
					Experimental	Predicted
1	0	0	0	0	69	66.85
2	0	2	0	0	54	60.87
3	0	0	0	0	65	66.85
4	0	0	2	0	88	86.54
5	2	0	0	0	30	34.04
6	0	0	0	0	68	66.85
7	0	0	−2	0	46	50.04
8	1	−1	−1	1	22	19.95
9	0	0	0	0	68	66.85
10	0	−2	0	0	17	12.70
11	−1	−1	1	−1	52	50.66
12	−1	−1	1	1	42	45.95
13	1	1	1	−1	76	75
14	1	−1	1	−1	42	40.29
15	1	1	−1	−1	60	52.12
16	1	1	−1	1	54	56.66
17	−1	1	−1	1	48	45.79
18	1	−1	−1	−1	30	29.16
19	0	0	0	0	66	66.85
20	1	1	1	1	87	82.29
21	0	0	0	2	60	55.20
22	0	0	0	0	67	66.85
23	0	0	0	0	65	66.85
24	−1	1	1	−1	64	62.12
25	−1	1	1	1	69	71.16
26	−2	0	0	0	35	33.54
27	−1	1	−1	−1	43	39.5
28	1	−1	1	1	29	33.83
29	−1	−1	−1	1	30	32.33
30	0	0	0	−2	48	55.37
31	−1	−1	−1	−1	39	39.79

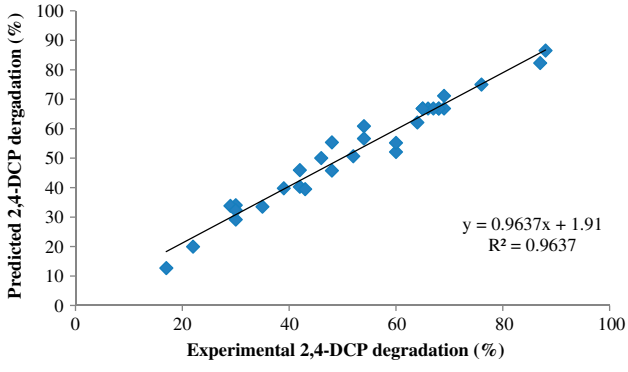

Fig. 1. Linear plot for actual vs. predicted biodegradation of 2,4-dichlorphenol.

Table 3
ANOVA for % degradation of 2,4-DCP

Source	DF	Seq SS	Adj MS	*F*	*P*
Regression	14	9,757.7	696.98	30.38	0
Linear	4	5,478.8	1,369.71	59.7	0
Square	4	3,400.5	850.11	37.05	0
Interaction	6	878.4	146.4	6.38	0.001
Residual error	16	367.1	22.94		
Lack-of-fit	10	352.2	35.22	14.23	0.002
Pure error	6	14.9	2.48		
Total	30	10,124.8			

$R^2 = 0.963$; $R^2_{adj} = 0.932$

Notes: DF—degree of freedom; Seq SS—sequential sum of squares; Adj MS—adjusted means square.

Table 4
Regression coefficient for 2,4-DCP biodegradation

Term	Coefficient	SE coefficient	T	P
Constant	−379.976	1.8105	36.928	0
X_1	81.6667	0.9778	0.128	0.9
X_2	9.17262	0.9778	12.316	0
X_3	−6.23214	0.9778	9.333	0
X_4	−16.2024	0.9778	−0.043	0.967
X_1^2	−8.26637	0.8957	−9.228	0
X_2^2	−0.30066	0.8957	−8.391	0
X_3^2	0.089658	0.8957	0.4	0.694
X_4^2	−11.5655	0.8957	−3.228	0.005
X_1X_2	1.1625	1.1975	4.854	0
X_1X_3	0.03125	1.1975	0.052	0.959
X_1X_4	−0.875	1.1975	−0.365	0.72
X_2X_3	0.29375	1.1975	2.453	0.026
X_2X_4	1.375	1.1975	2.871	0.011
X_3X_4	0.6875	1.1975	0.574	0.574

The interaction effect of temperature and ammonium sulphate concentration was shown in Fig. 2(c). The contour plot is also elliptical indicating the significant interaction between the variables. From the shape of the contour plot it could be seen that the effect of ammonium sulphate concentration is less significant on response compare to temperature. Using the middle point setting, the optimum setting for maximum degradation obtained was 35˚C for temperature and around 1.4 g L^{-1} for ammonium sulphate concentration. The contour plot for the interaction between the other variables was circle indicating the less significant effect on response. The model adequacy was also checked by analysing the internally studentized residuals data which shows that all the residuals, except three, have values under 2. The normality plot of residuals shows that all the residuals fall along the straight line (Fig. 3).

Using the desirability function the optimum values of experimental parameters obtained was: pH 7.36, temperature 35.1˚C, inoculum size 10 % (v/v) and $(NH_4)_2SO_4$ concentration 1.4 g L^{-1}. These optimum values were verified experimentally in batch mode using shake flask culture. The maximum of 100% degradation for 50 mg L^{-1} 2,4-DCP within 384 h was observed, which is up to 27% higher than unoptimized condition. Also the strain was able the degrade high concentration of 2,4-DCP up to 400 mg L^{-1} at optimized condition without much inhibition effect which used to prevail after 200 mg L^{-1} 2,4-DCP at unoptimized condition.

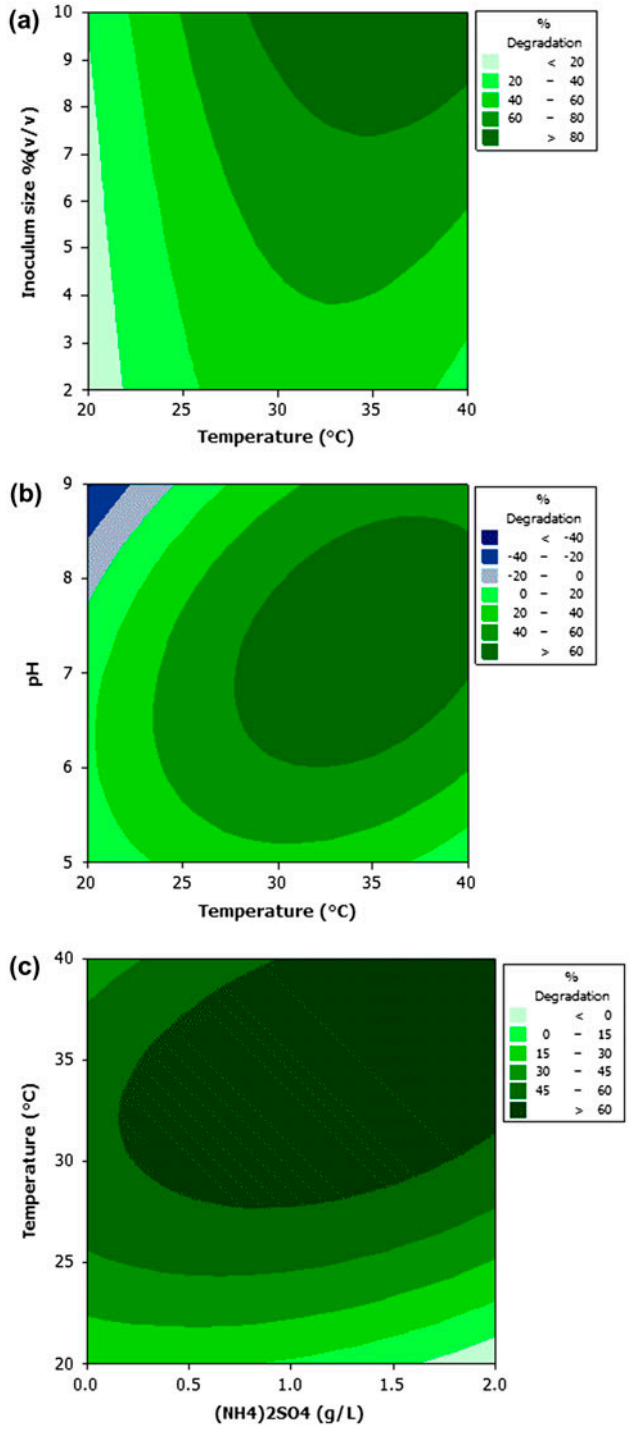

Fig. 2. (a) Contour plot showing the interaction effect of inoculum size % (v/v) and temperature (˚C) on 2,4-DCP, (b) Contour plot showing the interaction effect of temperature (˚C) and pH on 2,4-DCP and (c) Contour plot showing the interaction effect of $(NH_4)_2SO_4$ (g L^{-1}) and temperature (˚C) on 2,4-DCP.

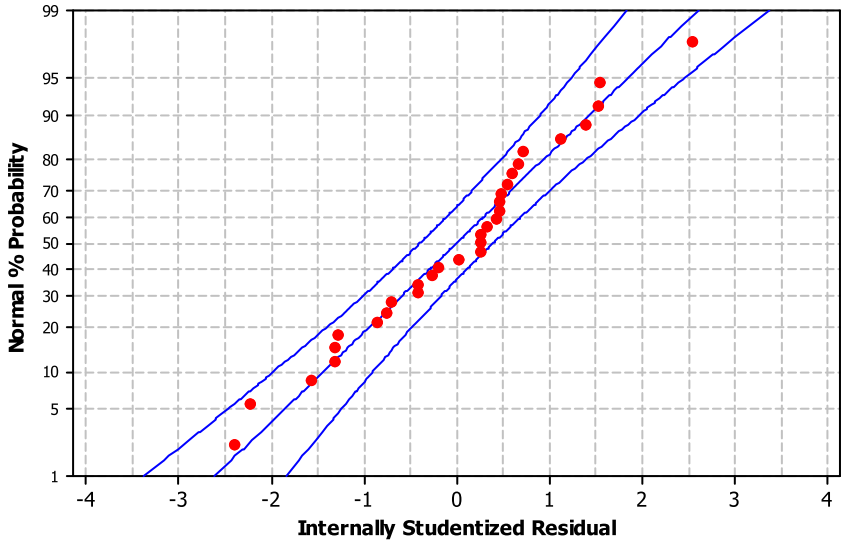

Fig. 3. Normal probability of internally studentized residuals for % biodegradation of 2,4-DCP.

3.2. Biodegradation kinetic of 2,4-DCP

The *B. endophyticus* strain CP1R strain has significantly degraded 2,4-DCP up to 400 mg L^{-1} at optimized condition. The degradation profile and residual concentration of 2,4-DCP was shown in Fig. 4. The isolate has completely degraded 25 and 50 mg L^{-1} of 2,4-DCP whereas, more than 75–80% degradation was observed for 100–400 mg L^{-1} 2,4-DCP at optimized culture condition. The relation between the 2,4-DCP removal rate and initial 2,4-DCP concentration was shown in Fig. 5. The plot is linear depicting that the 2,4-DCP removal rate increases almost linearly with initial 2,4-DCP concentration.

In this study, the higher removal rate of 2,4-DCP has been observed with increasing substrate concentration. This can be explained as the micro-organism use different bioenergetic strategies depending on the concentration and toxicity of 2,4-DCP. At lower concentration of 2,4-DCP, the micro-organism directs energy to the growth instead of biodegradation leading to less 2,4-DCP removal per cell. While in higher concentration the micro-organism directs energy to biodegradation instead of growth leading to higher DCP removal per cell. Such observation was also reported by Papazi and Kotzabasis that the degradation of high toxic dichlorophenol congener is higher as compared to low toxic dichlorophenol congener and monochlorophenol [22]. The micro-organism use a different bioenergetic strategy in such a way so that there is balance between the 2,4-DCP biodegradability, 2,4-DCP toxicity and growth.

The 2,4-DCP is toxic compound and has inhibition effect on micro-organism. The biodegradation kinetic for 2,4-DCP by *B. Endophyticus* strain CP1R was performed using the Andrew's substrate inhibition model as described by [23,24].

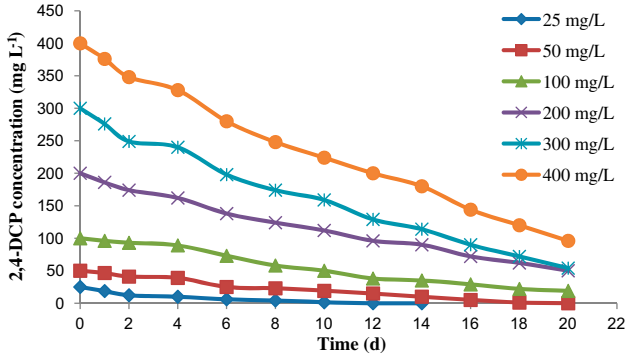

Fig. 4. 2,4-DCP degradation profile at different initial concentration by *B. endophyticus* CP1R.

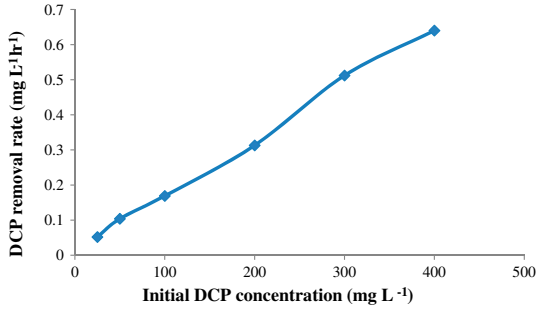

Fig. 5. Relation between 2,4-DCP removal rate and initial 2,4-DCP concentration.

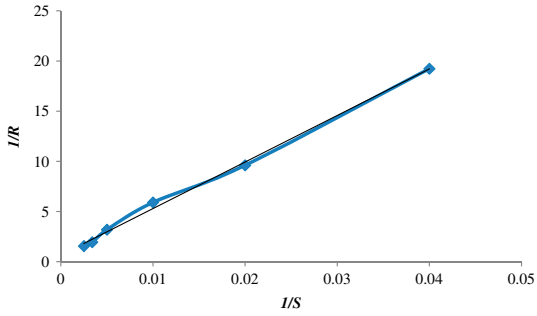

Fig. 6. Double reciprocal plot between $1/R$ vs. $1/S$.

$$R_s = \frac{R_m S}{K_s + S}\frac{K_{si}}{K_{si} + S} = \frac{R_m}{\left(1 + \frac{K_s}{S}\right)\left(1 + \frac{S}{K_{si}}\right)} \quad (4)$$

where R_s and R_m are the actual and maximum rate of 2,4-DCP degradation (mg DCP L^{-1} h^{-1}); S is the initial 2,4-DCP concentration (mg L^{-1}); K_s is the saturation constant (mg L^{-1}) and K_{si} is the 2,4-DCP inhibition constant (mg L^{-1}).

For low 2,4-DCP concentration or in present study as the inhibition effect was negligible up to 400 mg L^{-1} 2,4-DCP, the inhibition constant can be neglected, hence the Eq. (4) become as below:

$$R_S = \frac{R_m S}{K_s + S} \quad (5)$$

In the linear form,

$$\frac{1}{R_s} = \frac{1}{R_m} + \frac{K_s}{R_m}\frac{1}{S} \quad (6)$$

The plot between the $1/R_s$ vs. $1/S$ was plotted for experimental data (Fig. 6). The plot is linear with the slope of K_s/R_m and the intercept of $1/R_m$. From the best fit line the following values were obtained for bio-kinetic parameters:

R_m = 1.53 mg DCP h^{-1} L^{-1} and K_s = 711.5 mg L^{-1}

(R^2 = 0.997).

4. Conclusions

The degradation efficiency of 2,4-dichloropehnol by *B. endophyticus* strain CP1R was increased up to 27% at RSM optimized experimental conditions. Also the strain was able to utilize high concentration up to 400 mg L^{-1} of 2,4-DCP with minimal inhibitory effect at optimized condition. So the optimization of experimental parameters for maximizing the 2,4-DCP degradation using RSM was proven effective. The biodegradation kinetic of 2,4-DCP by *B. endophyticus* strain CP1R shows that the degradation of 2,4-DCP increases with increase in initial 2,4-DCP concentration up to 400 mg L^{-1}. The maximum biodegradation rate (R_m) and half saturation constant (K_s) obtained in this study were 1.58 mg DCP h^{-1} L^{-1} and 711.5 mg L^{-1}, respectively. The strain *B. endophyticus* CP1R shows good potential for treating the 2,4-DCP contamination in environment.

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