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Original article

Optimization of carbofuran insecticide degradation by Enterobacter sp. using response surface methodology (RSM)

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ABSTRACT

Response surface methodology and Plackett-Burman experiments were applied to optimize the biodegradation of carbofuran by using Enterobacter sp. stain BRC05 isolated from selected agricultural areas in peninsular Malaysia. The significant factors influencing the degradation of carbofuran were assessed using two-level Plackett–Burman Design (PBD) with five variables. Plakett Burman experiment showed that the following four variables were significant for carbofuran degradation including, carbofuran concentration, temperature, pH and nitrogen sources. Significant variables obtained in Plackett-Burman Design were further optimize using Central Composite Design (CCD). The outcome of the design for carbofuran degradation for each runs of the PBD experiment base on the design matrix, showed that the minimum and the maximum carbofuran degradation percentage were found to be 6.7% and 79.77% as presented in runs 4 and 1, respectively. Results obtained using Central Composite Design showed that the relations between the factors affect carbofuran degradation with significant response. The predicted results in CCD indicated that highest carbofuran degradation of 95.40% could be realized with carbofuran concentration of 92.50 mg/L, pH of 6.0, temperature 27.50 °C, nitrogen sources of 0.45 g/L and reaction period of 6 days. The predicted values were in agreement with the actual values with coefficient of determination with R^2 0.9719. Partial 16S rRNA sequence analysis showed that the carbofuran degrading isolate was closely related to members of the genera Enterobacter sp. The morphological and biochemical characteristics of the isolate also confirmed the phylogenetic signature. This study would provide an effective approach that could be beneficial for the bioremediation of carbofuran insecticide in polluted environment.

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1. Introduction

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl-methyl carbamate), a broad-spectrum carbamate insecticide (Mansano et al., 2018). The insecticide has been used widely by farmers to regulate many of insect pests of crops such as, tomatoes, corn, cabbages, potatoes and straw berries (Clasen et al., 2014). Carbofuran insecticide is harmful to the environment as well as human health (Begum and Vijayaraghavan, 2001; Fu et al., 2019). The carbofuran mechanism of action is mostly via reversible inhibition of acetyl cholinesterase enzyme (AChE) and subsequent endocrine disruption (Jemutai-Kimosop et al., 2014; Sakunthala Tennakoon et al., 2013).

The toxicity of the insecticide extends from human beings to both aquatic and terrestrial organisms with high sensitivity in fish and earthworms (WHO, 2010). Recently, more consciousness has been created on the negative effects of pesticides residues as well as the possibility of ground and surface waters pollution (Chin-Pampillo et al., 2015b). The high occurrence of carbofuran pollution, along with growing concern about the toxic effect of the compound, has prompted scientists to search for degradation alternatives for carbofuran contamination (Arraez-Roman et al., 2004; Devi and Iyer, 2017; Seo et al., 2007).

It has been known for several years that microbes are able to degrade pesticides and use them as source of energy for growth and metabolic activity (Chin-Pampillo et al., 2015a; Mohanta

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et al., 2012). Biodegradation of carbofuran by microorganisms is of particular interest since the conventional approaches used for mitigating the environment from this toxic compound are less effective, more cumbersome and costly (Yan et al., 2007). Several carbofuran degrading microorganisms have been isolated and characterized. Including strain of *Pseudomonas, Achromobacter, Sphingomonas, Novosphingobium and Rhodococcus* (Goncalves et al., 2006; Bano and Musarrat, 2004, Devi and Iyer, 2017; Karpouzas et al., 2000; Yan et al., 2007).

However, the literature on biodegradation efficiency of these bacteria in terms of complete removal of the compound are not enough in most cases (Xu et al., 2006; Yan et al., 2007). It is critical to isolate new bacterium which has much higher carbofuran degradation efficiency (Gongora-Echeverria et al., 2018). The toxic compounds degradation capacity of microbes is strongly affected by different factors such as nutrients and physicochemical conditions (Dzantor and Felsot, 1990; Park et al., 2006). Biodegradation using microorganisms depends not only on the availability of degradative microbes with suitable degradative enzymes, but also on a varied degree of environmental factors (Felsot et al., 1981; Tien et al., 2017). Optimizing those factors may increase the degradation capacity of the microbes. statistical models such as RSM, Plackett-Burman designs (PBD) and Central Composite design (CCD) can be used to optimize these parameters (Xia et al., 2012). In this study, a new carbofuran degrading bacteria was isolated from the agricultural soil polluted with the pesticide. The significant parameters influencing carbofuran biodegradation were identified, while the optimum levels of those variables for enhancing the carbofuran degradation by the bacteria was evaluated using Statistical models (Haddad et al., 2014; Popa Ungureanu et al., 2015).

2. Methodology

2.1. Chemicals and reagents

analytical standard Carbofuran PESTANAL@ 99% was purchased from Sigma Aldrich USA. High-performance liquid chromatography (HPLC) grade acetonitrile was purchased from Merch (Germany). All other chemicals and media ingredients used were of highest analytical grade are either from Merch (Germany), Sigma (India) or Fisher Scientific (Singapore).

2.2. Characterization of carbofuran degrading isolate

The bacteria were isolated in soil samples collected from different agricultural farms in peninsular Malaysia and used as initial inoculants for enrichment. Extraction of bacteria from the soil samples was done using serial dilution method as described by Collins and Lyne with little changes. One gram of each soil sample was suspended in 10 mL sterile medium (MSM). The MSM contained in g/L ($Na_2HPO_4{\cdot}12H_2O$ 6.5, KH_2PO_4 0.9, $FeSO_4{\cdot}7H_2O$ 0.01, $MgSO_4{\cdot}7H_2O$ 0.15 and 2 mL of trace element solution) supplemented with initial 5 mg/L of carbofuran as source of carbon (Collins et al., 1989). Other dilutions were prepared until a dilution of 10^{-10} was achieved. To ensure maximum extraction of the bacteria, the dilutions were agitated carefully. While for liquid culture experiments, 0.2 mL aliquots of appropriate dilutions of the soil samples were inoculated into MSM flask supplemented with 50 mg/L carbofuran and incubated at 32 °C agitated for 14 days in a shaker incubator at 150 rpm. The experiment was done in aseptic conditions in triplicate. Flask having the same inoculum without addition of carbofuran insecticide serves as control experiment. Using the spread plate as well as pour plates techniques, 0.2 mL aliquot of the incubated culture was pour in agar plates containing 50 mg/l carbofuran insecticide (Chaudhry and Ali, 1988). Using a sterile wire loop the bacterial colonies in the plates were then streaked into nutrient agar plates supplemented with 50 mg/l of carbofuran and incubated at 32 °C for two days. Sub-culturing was done every two weeks on carbofuran containing agar plates until pure colonies were obtained. Isolated single colonies differing in morphological features were then re-suspended in MSM and used for further studies.

2.3. Growth analysis of bacterial culture

Fresh grown culture of Enterobacter sp. was aseptically inoculated into ten ml of nutrient broth (NB) and incubated using shaker incubator at 140 rpm at 37 °C until the growth of 0.8–09 OD_{600nm} reached. The bacterial cell was then incubated for 24 h at 37 °C using shaker incubator at 150 rpm. The liquid culture was centrifuged at 10,000*x g* rpm for 10 min. (Chin-Pampillo et al., 2015a). The supernatant was carefully filtered and used for further studies (Ibrahim et al., 2015).

2.4. Optimization using response surface methodology (RSM)

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{11}X_1 + \beta_{22}X_2 + \beta_{33}X_3 + \beta_{44}X_4 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{14}X_1X_4 + \beta_{34}X_3X_4$$

Response surface methodology (RSM), which comprises regression analysis and factorial design, aids in measuring the significant parameters, in order to study the relations among the factors as well as choosing the optimal conditions of factors or appropriate responses (Ibrahim et al., 2015). In this study, two steps of optimization of carbofuran degrading strain were conducted by response surface methodology RSM (Ramasamy et al., 2017). Initial stage of the optimization was performed using Plackett–Burman Design (PBD) while, significant variables obtain were optimized further using central composite design (CCD) (Li et al., 2011). All tests were conducted in triplicate. (Chen et al., 2011). The percentage of carbofuran degradation was taken as the response (Rajendran et al., 2014). The correlation of the independent variables and that of the response were outline using the second order polynomial model that suits the response factors (El-Naggar Nel and Abdelwahed, 2014). The general formula for the second degree model applied is as follows:

In which the factor Y represent the response predicted while β_0, β_1, β_2, β_3, β_4, β_{11}, β_{22}, β_{33}, β_{44}, β_{12}, β_{13}, β_{23}, β_{24}, β_{14} and β_{34} are the regression coefficients of the model and is constant while β_0 represent the intercept, β_1, β_2, β_3, β_4 and β_{11}, β_{22}, β_{33} and β_{44} represent the linear as well as squared coefficients. While, X_1, X_2, X_3 and X_4 represent the independent factors. Factors combination (X_1, X_2) display the interface between the variables (Xia et al., 2012).

The factors that shows significant in Plackett Burman were further optimized by applying the CDD model (Yadav et al., 2015). The response design and the analysis of data was carried out with Design Expert software version 6.0.8 (See Table 1).

2.5. Statistical analysis

All the experiments were conducted in triplicate. Degradation studies were conducted using HPLC. All the data were statistically analyzed using Graph Pad v3.5. One-way ANOVA analysis of variance using the Tukey's test was carried out and $p < 0.05$ was considered statistically significant.

Table 1
Variables level affecting carbofuran degradation in Plackett-Burman design (PBD).

Parameters	Designation	Units	−1 (Low Actual)	+1(High Actual)
A	Carbofuran	Mg/L	5	200
B	Carbon S.	g/L	1	9
C	Nitrogen S.	g/L	0.1	0.8
D	Temperature	°C	15	40
E	pH	–	4	8

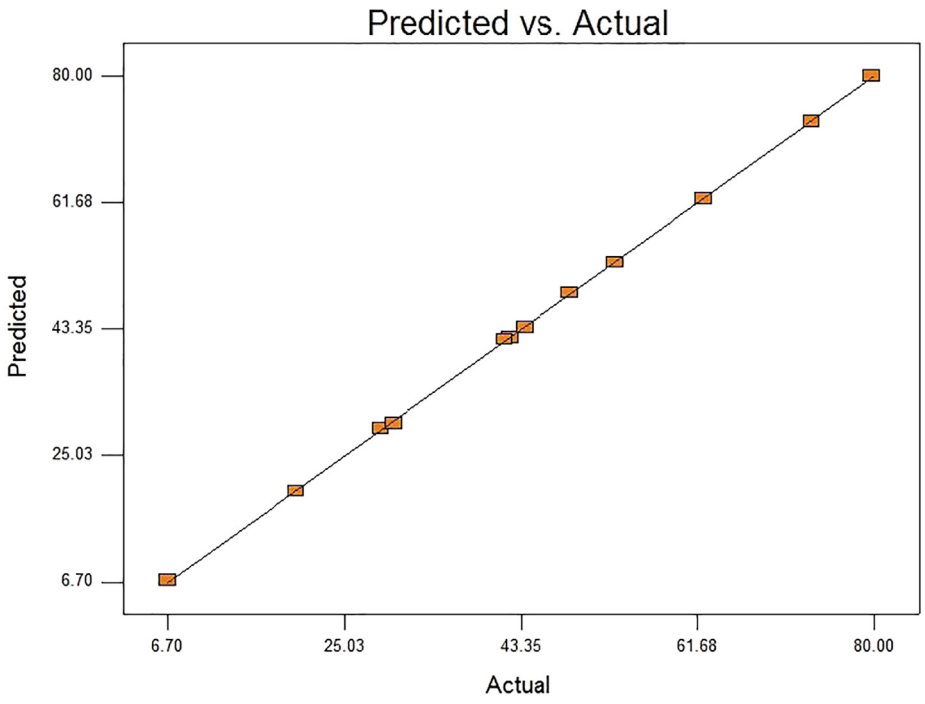

Fig. 1. Relationship plot showing interaction between predicted and the actual values of carbofuran content in Plackett–Burman.

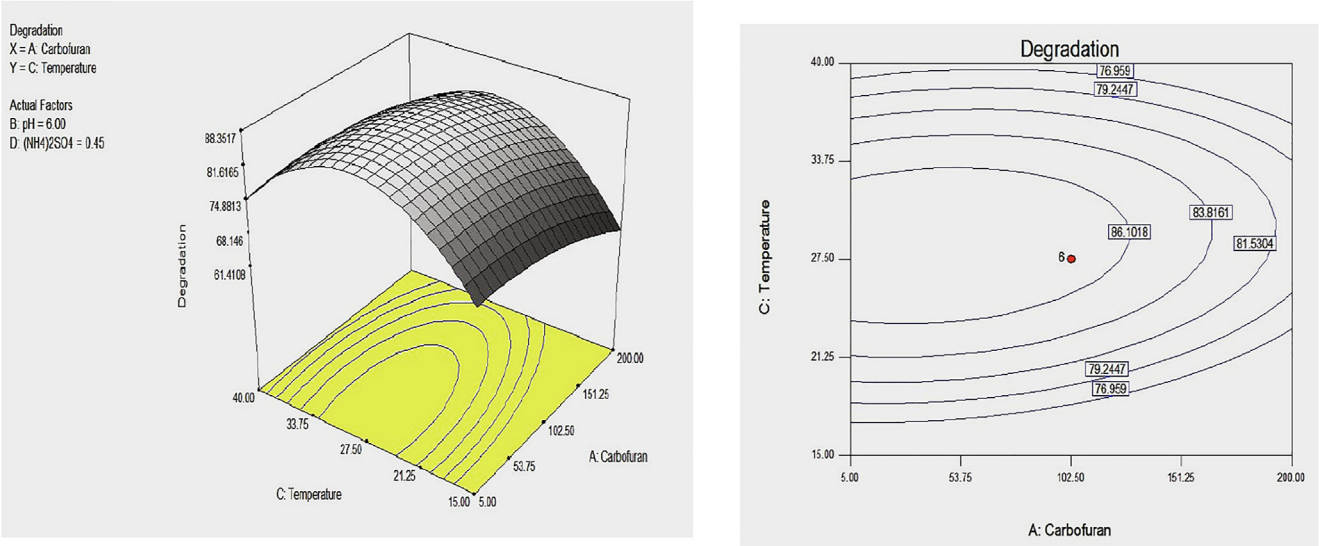

Fig. 2A. Model plot displaying 3D and 2D contour plotting for the effect of carbofuran and temperature on the degradation of carbofuran. by Enterobacter sp. Strain BRC05.

3. Results and discussions

Samples from different carbofuran polluted sites were collected and the enrichment technique using Mineral salts medium (MSM). In g/L (KH_2PO_4 0.9, $Na_2HPO_4{\cdot}12H_2O$ 6.5, $MgSO_4{\cdot}7H_2O$ 0.15, $FeSO_4{\cdot}7H_2O$ 0.02 and 2 mL of trace element solution). Morphologically distinct bacterial isolate was isolated. Growth of isolates was also monitored against variation in carbofuran concentration through change in cell biomass (OD_{600}). The bacterial strain has the capacity to grow on carbofuran media as carbon/nitrogen

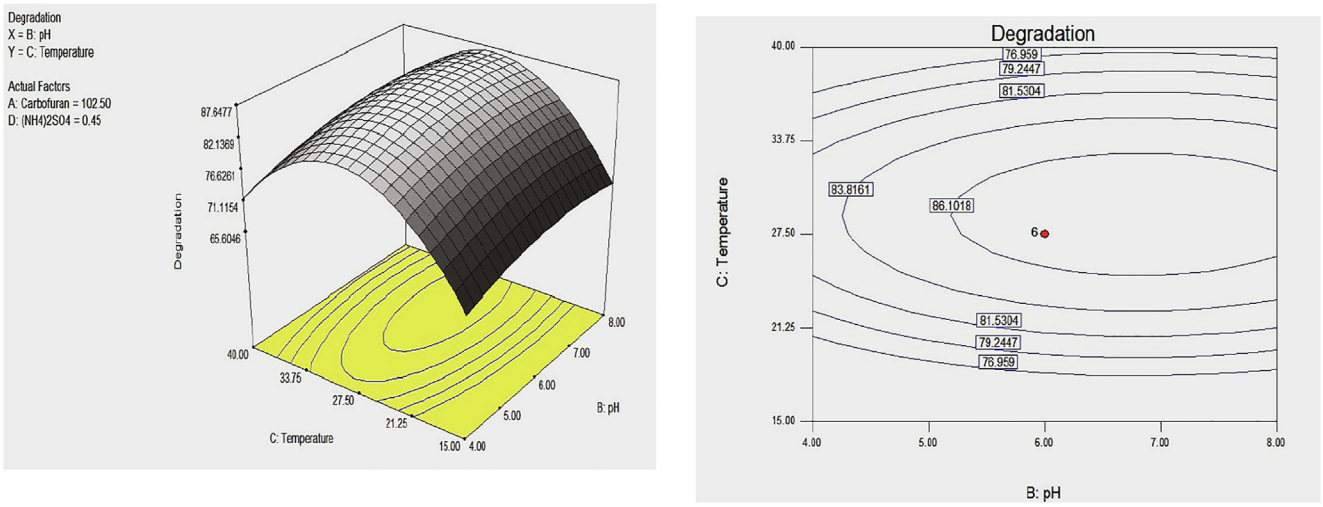

Fig. 2B. Represent the model plot displaying 3D and 2D contour plotting for the effect of pH and temperature on carbofuran degradation by Enterobacter sp. Strain BRC05. Value of factor carbofuran concentration as well as nitrogen source (NH_4Cl) was fixed at central point.

source and the strain was designated as BRC05. The bacterium was gram-negative motile, aerobic and was positive for oxidase, catalase and citrate. Its colony was milky-white. and can oxidize glucose with the production and maintenance of high concentration of acids as end products. The 16S rRNA gene sequence and phylogenetic analysis of the carbofuran degrader show resemblance to members of *Enterobacter sp* Figures 1 and 2). The strain morphological and biochemical characteristics also confirmed the phylogenetic signature. The ability of the isolates to utilize carbofuran as source of carbon was evaluated using HPLC and the reduction in the concentration of carbofuran was estimated against carbofuran standard curve (Tien et al., 2017). Pesticide biodegradation by bacteria has been reported by many researchers in various environments (Chin-Pampillo et al., 2015c; Khatun et al., 2018).

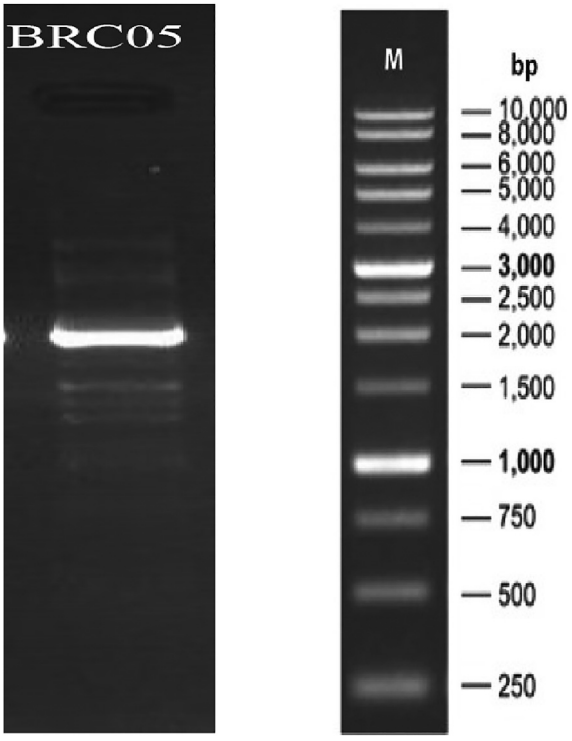

Fig. 3. Agarose gel electrophoresis of PCR product of 16S rRNA gene of strain BRC05.

Including soils, sediments, water bodies and sewage sludge and the capability of local carbofuran degrading bacteria were also reported (Devi and Iyer, 2017; Mohanta et al., 2012). These includes strains, such as *Achromobacter* sp., Sph*ingomonas* sp., *Flavobacterium, Paracoccus* sp., *Novo*sph*ingobium* sp. *and Alcaligenes faecalis,* among other (Bano and Musarrat, 2004; Devi and Iyer, 2017; Park et al., 2006; Yan et al., 2007). Bacteria use carbofuran through the hydrolysis of labile methylcarbamine linkage and producing carbofuran-7-phenol and methylamine as metabolites (Yan et al., 2007). Sphingomonas sp. strain SB5 was capable of degrading carbofuran to a number of metabolites including 2-hydroxy-3-(3-methylpropan-2-ol) phenol and some red intermediates (Park et al., 2006).

3.1. Plackett–Burman experiment

Enterobacter strain BRC05 is being optimized by applying response surface model to examine the best state for its capacity to degrade carbofuran. A total of 12 experimental designs were prepared by applying Placket-Burman design (PBD), comprising five independent parameters namely (A) carbofuran concentration, (B) pH, (C) temperature, (D) carbon source (glucose) and (E) nitrogen source (NH_4Cl). The higher and lower values of independent factors were selected according to previous literature. As shown in Table 2.

The PBD use the minimum number of runs to rapidly detect the variables with a significant effect on the response. Plackett-Burman design (PBD) deliver fast and efficient approach in identifying significant variables among greater amount of factors, thus, saves time and sustain extensive facts on each parameter. In Placket-Burman experiment, the main effects contain a complicated interaction with two factors associations (Yang et al., 2016). Therefore, the PBD is applied when studying major effects if the two way interactions are insignificant (Mirizadeh et al., 2014). Practically, PBD and fractional factorial designs were frequently applied to select significant variables that affect the process output measures or product quality (Sharma et al., 2009; Ibrahim et al., 2015; Ravanipour et al., 2015).

Experimental variables that affect carbofuran degradation are the temperature, pH, carbofuran concentration, carbon and nitrogen sources. The minimum and maximum values of the independent factors were chosen based on previous research (Khazaei et al., 2016; Kundu et al., 2016).

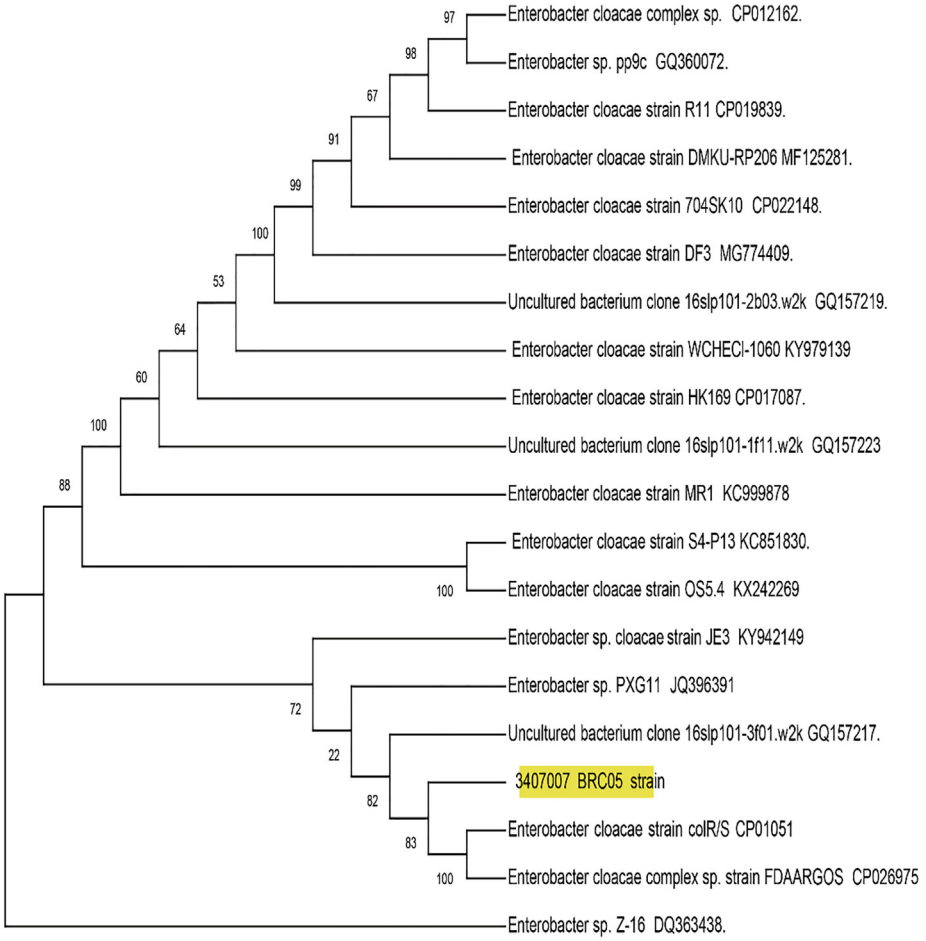

Fig. 4. Phylogenetic relationship using Neigbour-joining method cladogram showing relation between Enterobacter cloacae strain BRC05 and other related strains based on the 16S rRNA gene sequence analysis.

Table 2
The Placket-Burman experimental design on carbofuran degradation.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Run	A	B	C	D	E
1	200.00	8.00	15.00	9.00	0.80
2	5.00	4.00	40.00	9.00	0.80
3	200.00	4.00	40.00	9.00	0.10
4	5.00	4.00	15.00	1.00	0.10
5	5.00	4.00	15.00	9.00	0.80
6	5.00	8.00	15.00	1.00	0.10
7	5.00	8.00	40.00	1.00	0.80
8	200.00	8.00	40.00	1.00	0.80
9	200.00	4.00	15.00	1.00	0.80
11	5.00	8.00	40.00	9.00	0.10
12	200.00	4.00	40.00	1.00	0.10

Placket-Burman experiment confirms the result of the significant factors runs, which shows that among the variables used, carbon source concentration was insignificant factor for carbofuran degradation using isolate BRC05 an Enterobacter specie with p-value remained greater than 0.05. This might be due to the fact that the bacteria already using carbofuran as source of carbon instead of the additional source (glucose). While the remaining four factors, i.e. Nitrogen source (Ammonium sulphate), pH, initial carbofuran concentration and Temperature were found to be significant with p-values < 0.05. The model coefficient of determination (R^2) was 0.999 which may perhaps explain up to 99.9 percent of the response randomness. The Adjusted and the Predicted R-squared values were found to be 0.9987 and 0.9651 respectively, showing the existence of higher correlation between the actual and predicted values (See Tables 3 and 4).

3.2. Analysis using Central Composite design (CCD)

The optimum levels of the significant variables and the relations of these factors on the degradation of carbofuran were examined. Four significant variables achieved using Plackett–Burman design, were all experimented by central composite design. The levels selected for the variables were fixed according to previous studies (Ibrahim et al., 2015). The matrix for the Central Composite Design

Table 3
Results of placket-burman experimental design on carbofuran degradation.

Run	Factor 1 A	Factor 2B	Factor 3C	Factor 4 D	Factor 5 E	Response (%)
1	200.00	8.00	15.00	9.00	0.80	79.77
2	5.00	4.00	40.00	9.00	0.80	73.46
3	200.00	4.00	40.00	9.00	0.10	20
4	5.00	4.00	15.00	1.00	0.10	6.7
5	5.00	4.00	15.00	9.00	0.80	41.6
6	5.00	8.00	15.00	1.00	0.10	43.7
7	5.00	8.00	40.00	1.00	0.80	62.32
8	200.00	8.00	40.00	1.00	0.80	28.7
9	200.00	4.00	15.00	1.00	0.80	30.1
10	200.00	8.00	15.00	9.00	0.10	42.2
11	5.00	8.00	40.00	9.00	0.10	53.1
12	200.00	4.00	40.00	1.00	0.10	48.38

Table 4
The analysis of variance for Carbofuran degradation using Plackett-Burman Design (PBD).

Source	Sum of squares	DF	Mean square	F value	Prob > F	
Model	4987.77	10	498.78	879.66	0.0262	Significant
A	1378.94	1	1378.94	2431.95	0.0129	
6	961.57	1	961.57	1695.86	0.0155	
C	167.07	1	167.07	294.65	0.0370	
D	23.69	1	23.69	41.78	0.0977	
E	2053.37	1	2053.37	3621.41	0.0106	
AD	22.78	1	22.78	40.18	0.0996	
AE	126.78	1	126.78	223.60	0.0425	
BC	1844.30	1	1844.30	3252.68	0.0112	
BD	700.59	1	700.59	1235.59	0.0181	
CE	250.59	1	250.59	441.94	0.0303	
Residual	0.57	1	0.57			
Cor Total	4988.34	11				

Table 5
Response surface design using CCD and results for the optimization of carbofuran-degrading bacteria.

Run	A: Carbofuran	B: pH	C: Temp.	D:(NH4)2SO4	Experimental values	Predicted values
1	5.00	4.00	15.00	0.80	75.00	76.27
2	102.50	2.00	27.50	0.45	68.00	62.48
3	102.50	6.00	27.50	1.15	72.00	70.74
4	102.50	6.00	52.50	0.45	63.00	63.36
5	200.00	8.00	40.00	0.10	75.00	76.87
6	5.00	8.00	40.00	0.80	68.00	69.92
7	102.50	6.00	27.50	−0.25	71.00	71.68
8	200.00	8.00	15.00	0.80	71.00	71.15
9	200.00	8.00	15.00	0.10	68.50	66.16
10	102.50	6.00	27.50	0.45	50.00	51.78
11	200.00	4.00	15.00	0.10	72.00	72.54
12	102.50	10.00	27.50	0.45	68.64	64.56
13	200.00	4.00	40.00	0.80	68.50	70.60
14	102.50	6.00	27.50	0.45	64.00	63.06
15	5.00	8.00	15.00	0.80	74.00	77.32
16	5.00	8.00	40.00	0.10	75.00	76.20
17	102.50	6.00	27.50	0.45	86.00	83.04
18	5.00	4.00	40.00	0.10	65.43	68.12
19	102.50	6.00	27.50	0.45	74.00	74.06
20	297.50	6.00	27.50	0.45	82.00	81.67
21	102.50	6.00	27.50	0.45	19.00	23.75
22	5.00	8.00	15.00	0.10	41.00	35.98
23	92.50	6.00	27.50	0.45	95.00	95.40
24	5.00	4.00	40.00	0.80	91.00	90.34
25	102.50	6.00	27.50	0.45	87.00	87.09
26	5.00	4.00	15.00	0.10	82.00	87.09
27	102.50	6.00	2.50	0.45	88.54	87.09
28	200.00	8.00	40.00	0.80	91.00	87.09
29	200.00	4.00	40.00	0.10	85.00	87.09
30	200.00	4.00	15.00	0.80	89.00	87.09

showing actual and predicted values results is displayed in Table 5. CCD response were utilized to generate response surfaces as well as 2D and 3D contours so as to show the effects of these variables on cabofuran degradation. Response Surface Methodology-CCD assists in understanding how individual factors interact with each other (Roriz et al., 2009).

The outcomes of the analysis acquired from the CCD analysis were fitted in second order polynomial equation so as to define the carbofuran degradation dependence. The effects and interaction of independent variables were presented and also their response were predicted. 30 run experiments performed base on response surface methodology while the response (%) were shown as predicted and actual values. The lowest and highest carbofuran degradation percent were (95.00%) and (19%) respectively Table 5.

Analysis such as F-value, R-squared value and lack of fit were calculated so as to estimate the model. Analysis of variance (ANOVA) of the model shows that the model is highly significant, as it is evident from the F test with quite low probability value (<0.0001). Non significance of the lack of fit with a probability value greater than 0.1 is essential. The lack of fit test assesses the model failure to represent data in the experimental domain at points, which are not included in the regression. Therefore, the test is expected to be non-significant to signify the model (Ahmad et al., 2018). In this study, the non-significant value of lack of fit (F value 0.35) shows that the quadratic model is statistically significant for the response, and hence, it can be used for further analysis. (Mohajeri et al., 2010) reported that when the lack of fit is insignificant then the model is a good fit. Also, (Ibrahim et al., 2015) stated that if lack of fit is significant in a model, the model is weak to be used to predict the response. The lack of fit test indicates whether the chosen statistical model is adequate to define the observed data. The outcome of the response surface model indicates that A, B, C, A^2, B^2, C^2, BD were found to be the significant model terms (See Table 6).

The determination coefficients, R-squared and the adjusted R-squared value of the response model were 0.9719 and 0.9457 respectively, which signify that the correlation that exist between the predicted and actual values are very high. Also, the predicted R-squared was 0.9292 which is in agreement with the actual adjusted R-squared. Thus, this shows that the regression model offers an outstanding justification of the bond between the independent variables as well as the response. Adeq Precision calculate the signal to noise ratio in which a ratio of greater than 4 is desirable. In this study the ratio of 27.706 indicates an adequate signal. Indicating that the model can be used to navigate the design space. The coefficient of variation (CV) at minimum value is 5.03% showing that the model was accurate and reliable. The model's coefficient and their significances assessed using multiples linear regression for carbofuran degradation period. Analysis using multiple regression analysis by ignoring the values that are non-significant, shows the equation below as the basic quadratic model.

$$Y = 87.09 - 3.73\,A + 1.90\,B + 3.06\,C - -2.88\,A^2 - -2.31\,B^2 - -14.31\,C^2 + 2.98\,BD$$

in which Y represent response value (%), while (A, B C, and D) represent the coded levels of carbofuran concentration, nitrogen source, pH and temperature respectively (See Fig. 3).

The 2D and 3D contour plot signify graphical illustration of regression equation. It is aimed to know the interaction of parameters and trace the best level of each factor for maximum response (Li et al., 2011). Each plotted contour graph for carbofuran degradation indicates different collections of two test variables at a particular time, and retaining the other parameters at constant level (Ibrahim et al., 2015). The curved response surfaces indicate that there are distinct optimal parameters. If the surfaces are rather flat and symmetrical near the optimum, the optimized values may not vary widely from single parameter conditions (Kundu et al., 2016).

3.3. Interaction among factors

The model contour plots were presented to provide information on the response surface variables according to the second-order model through keeping two parameters to remain constant at zero and changing the last two parameters over the experimental range (Fig. 4). The interfaces between any of the two factors revealed that the values near the center point were best interacted. The shape of the contour plots revealed the interactive effects among the factors (See Figures 2A and 2B).

Circular pattern specified an insignificant interaction while an elongated response surface plot designated that the relationship between the subsequent variables were significant (Arasu et al., 2019; Xia et al., 2012). The nature of a contour plot specified weather the common interaction among the independent variables

Table 6
The analysis of variance (ANOVA) for central composite design.

Source	Sum of Squares	DF	Square	Mean Value	F Prob > F	
Model	6937.11	14	495.51	37.05	<0.0001	significant
A	333.76	1	333.76	24.96	0.0002	
B	86.79	1	86.79	6.49	0.0223	
C	224.24	1	224.24	16.77	0.0010	
D	38.41	1	38.41	2.87	0.1108	
A2	227.08	1	227.08	16.98	0.0009	
B2	145.86	1	145.86	10.91	0.0048	
C2	5613.61	1	5613.61	419.77	<0.0001	
D2	57.19	1	57.19	4.28	0.0563	
AB	41.09	1	41.09	3.07	0.1000	
AC	46.79	1	46.79	3.50	0.0811	
AD	0.35	1	0.35	0.026	0.8740	
BC	0.12	1	0.12	8.644E-003 0.9272		
BD	141.85	1	141.85	10.61	0.0053	
CD	14.75	1	14.75	1.10	0.3103	
Residual	200.60	15	13.37			
Lack of Fit	149.27	10	14.93	1.45	0.3561	Not significant
Pure Error	51.32	5	10.26			
Cor Total	7137.71	29				
Std. Dev.	3.66					
		R-Squared		0.9719		
Mean	72.65					
		Adj R-Squared		0.9457		
C.V.	5.03					
	Pred R-Squared			0.9262		
PRESS	933.72	Adeq Precision		27.706		

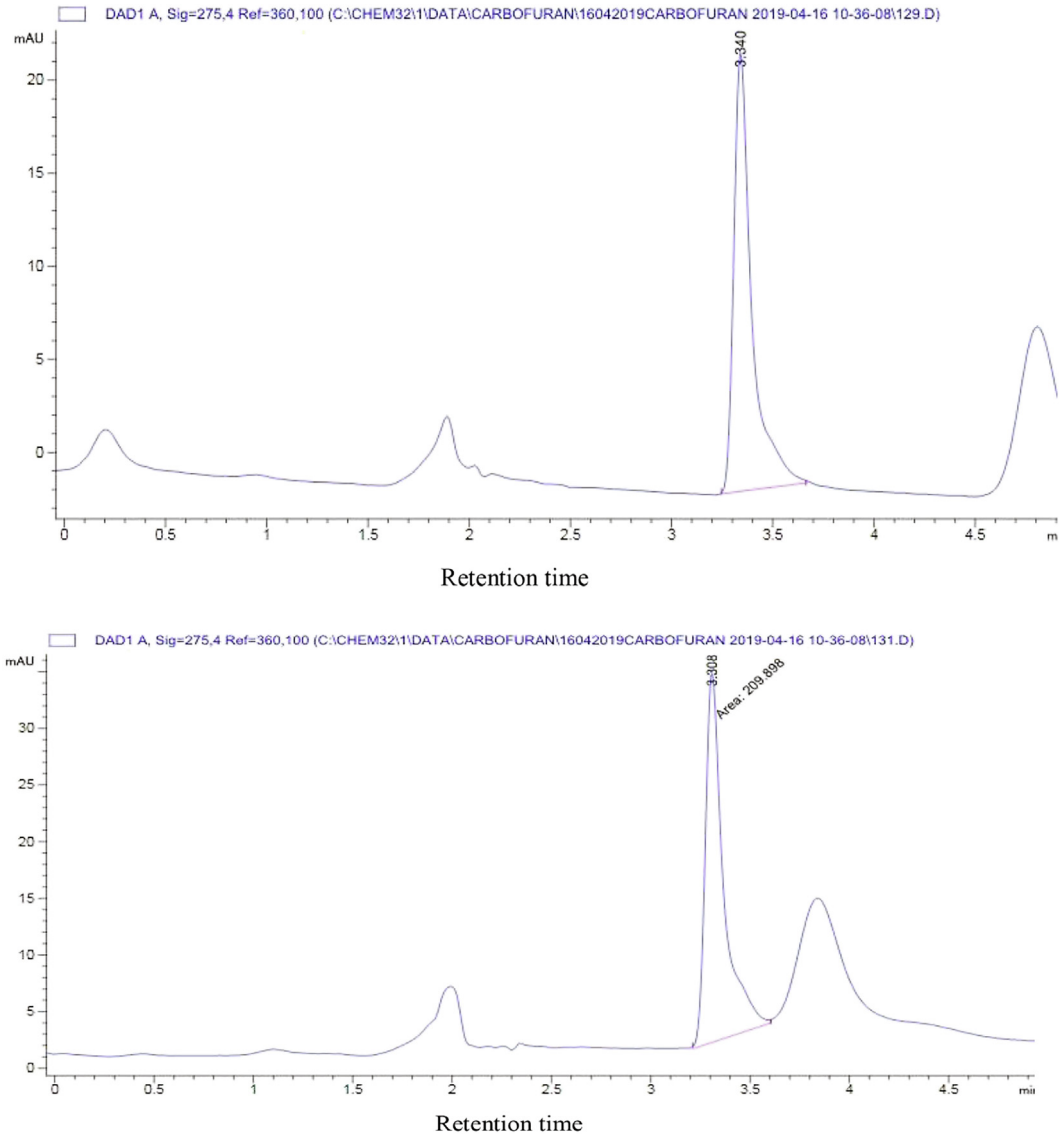

Fig. 5. Some Chromatographic profiles for biodegradation of carbofuran showing the carbofuran peak and its metabolites at various days of incubation.

were significant or not (Ahsan et al., 2017). The response surface for the degradation of carbofuran by enterobacter sp. showed a clear peak, meaning the optimal point was inside the design boundary level as Illustrated in Figs. 2A and 2B.

Interactive effect of temperature and initial carbofuran concentration on the degradation of carbofuran by Enterobacter sp. was shown in Fig. 2A. It was observed that carbofuran removal ability increased at optimum conditions of temperature or initial carbofuran concentration and degradation rate reduced with a further increase with the temperature or initial carbofuran concentration. Also, the interaction between temperature and pH value was presented in Fig. 2B. The elliptical plot showed that the degradation ability of carbofuran was greatly affected by the change in pH or temperature. The decline in carbofuran was increased with increasing temperature. After the optimum temperature, the biodegradation ability decrease as the temperature was further increased (See Fig. 5).

4. Conclusion

Highly effective carbofuran-degrading Enterobacter sp. strain BRC05, was isolated from agricultural soil using enrichment method. A powerful method of optimization using response surface methodology was applied to define the effects of four variable factors. (pH, nitrogen source, temperature and initial carbofuran concentration) as well as their interaction on carbofuran degradation. Carbofuran degradation efficiency could be maximized up to 95% degradation under the following conditions: carbofuran concentration 92.50 mg/L, temperature of 27.50 °C, pH of 6 and nitrogen source 0.45. The similarity between the predicted and the experiential results has confirmed the validity and applicability of RSM-CCD model in the optimization processes. The results obtained recommend that statistical optimization approach is an efficient means to predict the biodegrading activity of pesticides. The newly isolated Enterobacter sp. BRC05 will be potentially beneficial in substantial application for carbofuran bioremediation and remediation of other pesticide polluted soils.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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