Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 4(2) March 2026
DOI: https://dx.doi.org/10.4314/jobasr.v4i2.7

Enhanced Biodegradation of Carbofuran by *Pseudomonas* sp. Strain SAB2 Through Multivariate Process Statistical Optimization

Ahmad Umar Bello[1], Jamila Mashi Ahmed[2], Kamaluddeen Bababgana[3], Uwais Muhammad Atiku[4], Kabiru Yakubu[5], Aminu Yusuf Fardami[6], Abba Babandi[1,7] and Salihu Ibrahim[1,2,8*]
[1,2,3]Department of Biochemistry, Faculty of Basic Medical Sciences, Bayero University, Kano, PMB 3011, Kano, Nigeria.
[4]Centre for Biotechnology Research, Bayero University, Kano, PMB 3011, Kano, Nigeria.
[5]Department of Botany, Faculty of Science, Adamawa State University PMB 25 Mubi Adamawa State Nigeria
[6]Department of Environmental Health Science, Faculty of Allied Health Sciences, Bayero University, Kano, PMB 3011, Kano, Nigeria
[7]Medical Biochemistry Unit, Faculty of Basic Medical Sciences, Federal University Dutse, PMB 7156, Dutse, Jigawa State-Nigeria
[8]Department of Biochemistry, Sciences, Sa'adu Zungur University PMB 65 Gadau Bauchi, Nigeria
[*]Corresponding Author Email: sibrahim.cbr@buk.edu.ng

ABSTRACT
Carbofuran is a N-methyl carbamate pesticide that is used for pest control in many countries including Nigeria. The pesticide has been classified as acutely toxic and as such its persistence in the environment for a long time can be detrimental to the environment. *Pseudomonas* sp. strain SAB2 (PX375346) was isolated and identified from contaminated farmland soil in research conducted previously. A statistical optimization tool was used to optimize the degradation efficiency of strain SAB2. The degradation efficiency of the isolate is determined by the various factors affecting its growth and as such Plackett–Burman Design was applied in other to limit the factors to only significant in other to manage resources, PBD was able to identify the factors: temperature, pH, inoculum size, and carbon source as significant factors affecting the isolate`s degradation efficiency. The optimum temperature, pH, inoculum size and carbon source (sucrose) found was: 38°C, 6, 0.5% and 1.25 g/L respectively. Second order polynomial regression model showed the best fit to the experimental data with an R^2 value (0.9907), adjusted R^2 (0.9821), predicted R^2 (0.9547) and F value (114.61) which means the model has strong predictive capability. The results found demonstrate the metabolic capability of *Pseudomonas* sp. strain SAB2 for carbofuran biodegradation. This study highlights the promise of strain SAB2 as a candidate for environmentally sustainable remediation of carbofuran-contaminated sites and provides a framework for further scale-up and field-level application.

Keywords:
Carbofuran;
Biodegradation;
Pseudomonas sp. strain SAB2;
Response Surface Methodology

INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) can be classified as non-selectively N-methyl carbamate pesticide that is used extensively in agricultural practices as a form of pest control (Ariffin & Rahman, 2020). It is a highly effective systemic insecticide as has been used for produce like: maize, rice, vegetables, and fruits. Its mechanism of action is such that it inhibits the enzyme acetylcholinesterase and such action stops the breakdown of acetylcholine and that leads to its continuous build-up leading to various neurological symptoms like: paralysis, spasms and death (Randika et al., 2022). Although carbofuran is effective against target pests the down side of it broad spectrum nature can lead to undesirable consequences such as affecting birds, fish, mammals and also humans which lead to some developed countries to ban its use (Bello et al., 2026).
Carbofuran is still be extensively used in a lot of

How to cite this article: Ahmad Umar Bello, Jamila Mashi Ahmed, Kamaluddeen Bababgana, Uwais Muhammad Atiku, Kabiru Yakubu, Aminu Yusuf Fardami, Abba Babandi & Salihu Ibrahim (2026). Enhanced Biodegradation of Carbofuran by *Pseudomonas* sp. Strain SAB2 Through Multivariate Process Statistical Optimization. *Journal of Basics and Applied Sciences Research*, *4*(2), 56-66. https://dx.doi.org/10.4314/jobasr.v4i2.7

developing countries and as such this has caused significant environmental contamination leading to ecosystem disruption (Bello et al., 2026). The extensive usage of carbofuran has led to its persistence in the environment and as such its residues have been found in soils, waterways and agricultural crops and as such levels that can be detrimental to ecosystems and humans (Bello et al., 2026; Mishra et al., 2020). The already established methods of carbofuran remediation such as: adsorption, incineration, and chemical oxidation are effective but with a downside of being expensive, energy-intensive, and may generate secondary pollutants. Consequently, biological remediation strategies have been developed and are sustainable and environmentally friendly alternative for detoxification of carbofuran-contaminated environments (Kaur & Balomajumder, 2020).

The Bacterial genus *Pseudomonas* have been reported for its ability to degrade a wide range of compounds including pesticides like carbamates (Bello et al., 2026; Mishra et al., 2020). They possess the ability to utilize carbofuran as either sole carbon/nitrogen source for its metabolism and growth and in the process degrade it into less harmful compounds (Zhang *et al.,* 2020). The efficiency of bacterial degradation is influenced by the levels of factors that have been found to influence their growth such as: temperature, pH, inoculum size, incubation time, initial substrate concentration, and nutrient availability. Optimization of these factors is therefore critical to enhance biodegradation efficiency and to enable potential large-scale applications (Ibrahim et al., 2016a; Ibrahim et al., 2016b)

Traditional optimization approach like one-factor-at-a-time (OFAT) are time-consuming, resource intensive and fail to account for interactive effects between variables (Ibrahim et al., 2020a). In other to improve on its lapses statistical optimization technique for optimization like Response Surface Methodology (RSM) was developed and has a robust statistical and mathematical framework for evaluating multiple variables simultaneously and determining their optimal levels with reduced experimental runs while simultaneously taking into account potential interactive effects (Bas & Boyaci, 2007; Ibrahim *et al.,* 2020b). Screening designs such as Plackett–Burman Design (PBD) are particularly useful for identifying significant factors among many variables (Plackett & Burman, 1946), while Central Composite Design (CCD) enables modeling of quadratic and interaction effects to develop predictive second-order polynomial models. RSM has been successfully applied in optimizing various biodegradation processes (Ibrahim et al., 2015; Lukman et al., 2024).

In a previous study, a carbofuran-degrading bacterium identified as *Pseudomonas* sp. strain SAB2 (GenBank accession no. PX375346) was isolated from carbofuran contaminated agricultural soil. The strain was found to be effect in degrading carbofuran and as such the degradation efficiency of the isolate is needed to be maximized by statistical optimization process.

Therefore, the present study aimed to optimize carbofuran-degrading parameters of *Pseudomonas* sp. strain SAB2 using a two-stage statistical approach involving Plackett Burman Design for significant factor screening followed by Central Composite Design under Response Surface Methodology. The study further evaluated model adequacy and validated the optimized conditions, with the ultimate goal of enhancing carbofuran biodegradation efficiency for potential environmental bioremediation applications.

MATERIALS AND METHODS

Chemicals and Reagents

Analytical-grade carbofuran (purity ≥ 98%) was procured from Sigma Aldrich (USA). All other chemicals and reagents used in this study were of analytical grade. Nutrient agar (NA), nutrient broth (NB), and mineral salt medium (MSM) components were obtained from standard microbiological suppliers. Stock solutions of carbofuran were prepared in sterile distilled water and stored at 4°C until use.

Bacterial Strain and Maintenance

The carbofuran-degrading bacterium used in this study, *Pseudomonas* sp. strain SAB2 (GenBank accession no. PX375346), was previously isolated from agricultural soil contaminated with pesticide residues. The strain was maintained on nutrient agar slants at 4°C and subcultured periodically to ensure viability. For experimental purposes, a loopful of the culture was inoculated into nutrient broth and incubated at 30–37°C (as applicable) for 18–24 h to obtain an actively growing culture. For long term storage, the isolated carbofuran-degrading bacterium was maintained on well labelled slant and stored at 4^{o}C for routine use or stored at -20 °C in Microbank^{TM} (PRO-LAB Diagnostics) vials (beads in a cryopreservative fluid) for long term-storage.

Preparation of Inoculum

An overnight culture of *Pseudomonas* sp. SAB2 was centrifuged at 5000 rpm for 10 min, washed twice with sterile saline solution (0.85% NaCl), and resuspended in sterile distilled water. The optical density (OD) was adjusted to approximately 0.5 at 600 nm, corresponding to ~1.0×10^8 CFU/mL, and used as the standardized inoculum for degradation experiments.

Mineral Salt Medium (MSM) Composition

The biodegradation experiments were conducted in mineral salt medium (MSM) containing (per liter): KH_2PO_4 (1.0 g), K_2HPO_4 (1.0 g), NH_4NO_3 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $CaCl_2$ (0.02 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), and trace elements solution (1 mL) (Thongmee and Sukplang 2024). The pH of the medium was adjusted to

7.0 before sterilization at 121°C for 15 min. Carbofuran was added aseptically to the sterilized medium to achieve the desired concentration.

Experimental Design for Optimization

Plackett-Burman Design

Plackett-Burman Design was employed to screen significant variables affecting carbofuran bacterial growth and degradation by the utilization of 12 experimental runs. The parameter levels were fixed in accordance to the work of Fareed *et al.* (2017). Selected independent variables included temperature (32 - 38°C), pH (6 - 8), inoculum size (0.5 - 1.5), carbofuran concentration (40 - 70 mg/L), carbon source (0.75 - 1.25), and additional nutrient sources (0.75 - 1.25). Each variable was evaluated at two levels (high and low), and experimental runs were generated using Design-Expert® software (version 13.0.5).

Optimization Using Central Composite Design (CCD)

Central composite design is employed if the relationship between two independent variables need to be studied on how they collectively affect the response variable (Babandi et al., 2021). Significant factors identified from PBD were taken a step further to be optimized using CCD under the Response Surface Methodology (RSM). CCD uses the significant factors identified previously with five levels of the factors, which are; low (-1), high (+1), center (0), axial low and high (-±α). The experimental matrix was experimentally tested after which result with p-value <0.05 were considered to be statistically significant. Data were fitted to a second-order polynomial equation for each dependent variable *Y* (Equation 1) (Lukman et al., 2024; Ibrahim *et al.*, 2020b). A second-order polynomial equation was used to describe the relationship between independent variables and carbofuran bacterial growth was used as the response variable, *Y*.

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

where *Y* is the predicted response parameter, β_0, is the constant regression coefficient of the model, β_i is the linear coefficient term, β_{ii} is the squared coefficients, β_{ij} is the interaction coefficients, and x_i and x_j are the independent parameters. The experimental design and the statistical analysis of the data was then analyzed using Design-Expert version 13.0.5. Experimental runs were conducted in triplicate, and the adequacy of the model was evaluated using analysis of variance (ANOVA), coefficient of determination (R^2), adjusted R^2, predicted R^2, lack-of-fit test, and adequate precision.

Model Validation

To validate the developed model, experiments were performed under predicted optimal conditions, and the experimental degradation efficiency was compared with the model-predicted value. The closeness between predicted and observed results confirmed model reliability.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical analyses were performed using Design-Expert® software. Significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Screening of Significant parameters Using Plackett-Burman Design

A 12 experimental matrix was conducted using stated parameters in PBD as shown in Table 1. The experimental results demonstrated noticeable variation in the bacterial growth efficiency across different combinations of tested factors, indicating that the process is strongly dependent on environmental and nutritional conditions. The highest growth was observed at run 3 with an OD600nm of 0.56 while the lowest growth was found at run 5 with an OD600nm of 0.27. This result indicates that higher level of the factor tested stimulated the organism to have a higher growth while lower levels of tested factors resulted in lower growth. This result is in contradiction with the work of Mustapha *et al.* (2020) which found that medium level of factors provided the highest growth and that could be attributed to the fact that different bacteria, isolates were used and as such they likely possess distinct metabolic capability.

Table 1: Biodegradable Effect of *Pseudomonas* sp. Strain SAB2 on a Key Determinants

Run	A	B	C	D	E	F	Bacterial growth (OD 600nm)
1	32	8	1.5	40	1.25	1.25	0.51
2	32	6	1.5	40	1.25	1.25	0.40
3	37	8	0.5	70	1.25	1.25	0.56
4	32	8	1.5	70	0.75	0.75	0.43
5	32	6	0.5	40	0.75	0.75	0.27
6	37	8	0.5	40	0.75	1.25	0.48
7	37	6	1.5	70	1.25	0.75	0.51
8	37	6	0.5	40	1.25	0.75	0.41
9	32	6	0.5	70	0.75	1.25	0.30

10	37	8	1.5	40	0.75	0.75	0.55
11	32	8	0.5	70	1.25	0.75	0.43
12	37	6	1.5	70	0.75	1.25	0.48

A = Temperature (°C), B = pH, C = Inoculum size (% v/v), D = Initial carbofuran concentration (mg/L), E = Carbon source (g/L), F = Nitrogen source (g/L)

In this study, the PBD analysis of variance results (Tables 2) revealed distinct sets of significant factors for bacterial growth. Statistical analysis revealed that temperature, pH, inoculum size, and carbon source significantly influenced *Pseudomonas* sp. strain SAB2 growth efficiency and degradation ($p < 0.05$), whereas, initial carbofuran concentration and nitrogen source concentration showed comparatively lower effects (Tables 2). The positive regression coefficients observed for temperature and inoculum size suggest that increased metabolic activity and higher biomass concentration enhanced carbofuran utilization. Conversely, elevated carbofuran concentration exhibited a negative coefficient, indicating possible substrate inhibition at higher levels. This result is in contradiction to the work of Mustapha *et al.*(2020) which identified both nitrogen source and carbofuran concentration as significant factors while temperature was not significant.

Table 2: Analysis of variance for quadratic model of strain SAB2 bacterial growth for carbofuran degradation from PBD

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.0899	9	0.0100	51.46	0.0192	Significant
A	0.0229	1	0.0229	118.17	0.0084	
B	0.0190	1	0.0190	97.99	0.0101	
C	0.0109	1	0.0109	55.90	0.0174	
D	0.0005	1	0.0005	2.38	0.2626	
E	0.0036	1	0.0036	18.62	0.0497	
F	0.0012	1	0.0012	6.25	0.1296	
AB	0.0001	1	0.0001	0.2893	0.6445	
AF	0.0000	1	0.0000	0.0890	0.7936	
DE	0.0003	1	0.0003	1.43	0.3538	
Residual	0.0004	2	0.0002			
Cor Total	0.0903	11				
R^2	0.9957					
Adj R^2	0.9764					
Adeq. Precision	22.6561					

A = Temperature (°C), B = pH, C = Inoculum size (% v/v), D = Initial carbofuran concentration (mg/L), E = Carbon source (g/L), F = Nitrogen source (g/L)

The model's F-value (51.46) and low p-value (0.0192) confirmed the statistical significance of the screening model. The coefficient of determination (R^2) indicated good agreement between experimental and predicted values, demonstrating the suitability of PBD for identifying critical parameters prior to optimization. The model revealed that initial carbofuran concentration and nitrogen source were not significant parameters for *Pseudomonas* sp. strain SAB2 for carbofuran degradation, which is contrary to the work of Mustapha *et al.* (2020) which found that both initial carbofuran concentration and nitrogen source were significant factors affecting the degradation efficiency of the isolate. Figure 1 shows the similarity plot between the predicted and the actual value obtained in the PBD for SAB2, which can be expressed as Equation 2 and 3 respectively.

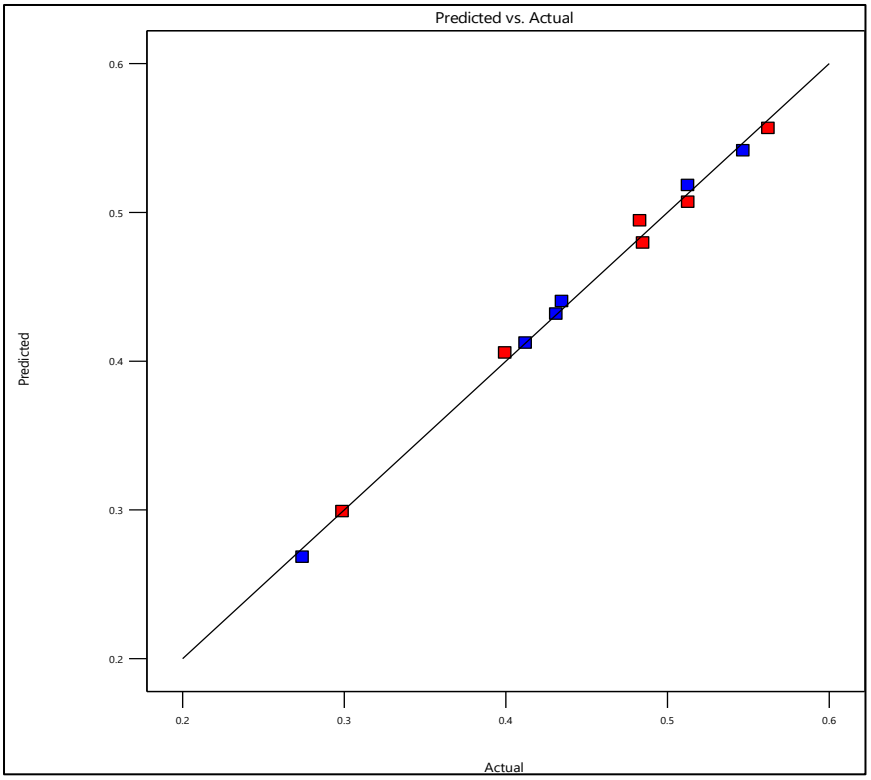


Figure 1: Similarity plot of predicted vs actual values of bacterial growth for *Pseudomonas* sp. Strain SAB2.

Coded Equation of PBD for *Pseudomonas sp* Strain SAB2

Y = 0.446125 + 0.051275 * A + 0.0466917 * B + 0.0368333 * C + 0.00728333 * D + 0.0237667 * E + 0.0150833 * F + -0.003975 * AB + -0.0018 * AF + 0.00885 * DE (2)

Actual Equation of PBD for *Pseudomonas sp* Strain SAB2

Y = -1.19763 + 0.03452 * A + 0.101547 * B + 0.0736667 * C + -0.00187444 * D + -0.0347333 * E + 0.159693 * F + -0.00159 * A * B + -0.00288 * A * F + 0.00236 * D * Carbon source (3)

Optimization of Significant Factors Using Central Composite Design

The significant variables identified by PBD were further optimized using Central Composite Design (CCD) under Response Surface Methodology. The experimental results obtained from CCD showed substantial improvement in *Pseudomonas* sp. Strain SAB2 growth efficiency and carbofuran degradation compared to non-optimized conditions. The results obtained from the central composite design experimental matrix was found to fit the second order polynomial equation and could explain the dependence of carbofuran degradation on the various factors. Through the use of RSM, the effects of the independent factors and their interaction were represented and their response was predicted. Table 3 shows the experimental design and result of CCD on carbofuran degradation by strain SAB2.

Table 3: Experimental design and result of CCD on carbofuran degradation by strain SAB2

Run	A	B	C	D	Experimental value	Predicted value
1	32	6	1.50	1.25	0.56	0.56
2	35	7	1.00	1.00	0.36	0.37
3	38	8	0.50	0.75	0.58	0.58
4	32	6	1.50	1.25	0.44	0.42
5	37	6	1.50	1.25	0.49	0.49
6	35	5	1.00	1.00	0.57	0.57
7	35	7	1.00	1.00	0.44	0.46

8	35	7	1.00	1.00	0.59	0.61
9	35	7	2.00	1.00	0.54	0.53
10	35	9	1.00	1.00	0.40	0.41
11	32	8	0.50	0.75	0.50	0.49
12	32	8	0.50	1.25	0.52	0.52
13	32	8	1.50	0.75	0.54	0.54
14	38	6	0.50	1.25	0.60	0.60
15	38	8	0.50	1.25	0.42	0.42
16	30	7	1.00	1.00	0.49	0.49
17	35	7	1.00	1.00	0.51	0.51
18	38	6	0.50	1.25	0.48	0.49
19	35	7	1.00	1.00	0.47	0.47
20	35	7	0.50	1.00	0.58	0.58
21	35	7	1.00	0.50	0.48	0.47
22	37	8	1.50	1.25	0.46	0.46
23	38	6	1.50	0.75	0.56	0.55
24	35	7	1.00	1.50	0.59	0.59
25	32	6	0.50	1.25	0.51	0.51
26	38	8	1.50	0.75	0.45	0.46
27	35	7	1.00	1.00	0.50	0.49
28	32	8	1.50	1.25	0.46	0.47
29	40	7	1.00	1.00	0.49	0.49
30	32	6	0.50	0.75	0.46	0.46

A = Temperature (°C), B = pH, C = Inoculum size (% v/v), D = Carbon source (g/L)

Analysis of variance (ANOVA) for the quadratic polynomial model shows that generated to predict degradation efficiency was statistically significant ($p < 0.05$), with a high R^2 value, indicating strong correlation between predicted and experimental responses. The adjusted R^2 and predicted R^2 values were in reasonable agreement, confirming model reliability. More so, the difference between R^2 predicted Bacterial growth and R^2 adjusted bacteria growth is within 0.2, affirming the suitability of the model. Furthermore, the non-significant lack-of-fit ($p > 0.05$) demonstrated that the model adequately described the experimental data (Table 4). Babandi *et al.* (2021) reported that if the lack of fit of a model is found to be significant then it cannot be used to predict the response variable. The *p*-value shows that this model for the response is statistically significant with the prob > F value for the model parameter that is < 0.05. The result obtained shows that C, D, AB, AD, BD, CD, A^2, B^2, C^2 and D^2 were significant model terms.

Table 4: Analysis of variance for quadratic model of strain SAB2 bacterial growth for carbofuran degradation from CCD

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Model	0.1057	14	0.0076	114.61	< 0.0001	Significant
A	0.0001	1	0.0000	0.2405	0.631	
B	0.0002	1	0.0000	0.3858	0.5438	
C	0.0057	1	0.0057	86.91	< 0.0001	
D	0.0221	1	0.0221	335.09	< 0.0001	
AB	0.0111	1	0.0111	169.02	< 0.0001	
AC	0.0003	1	0.0000	0.6763	0.4237	
AD	0.0067	1	0.0067	101.75	< 0.0001	
BC	0.0001	1	0.0001	1.09	0.313	
BD	0.0007	1	0.0007	11.33	0.0042	
CD	0.0245	1	0.0245	372.11	< 0.0001	
A^2	0.0219	1	0.0219	332.87	< 0.0001	
B^2	0.0018	1	0.0018	27.48	< 0.0001	
C^2	0.0053	1	0.0053	80.72	< 0.0001	

D^2	0.0011	1	0.0011	16.27	0.0011	
Residual	0.001	15	0.0001			
Lack of Fit	0.0008	10	0.0001	1.98	0.2329	Not Significant
Pure Error	0.0002	5	0.0000			
Cor Total	0.1067	29				
R^2	0.9907					
Adj. R^2	0.9821					
Pred R^2	0.9547					
Adeq. Presidion	41.85					

(4)

Adequate precision measures the signal to noise ratio which should be > 4 is advantageous (Ibrahim et al., 2015). The ratio of 41.85 signifies that the models could be used to predict the response, while the coefficient of variation (CV) at lower value showed that the experiment were precise and reliable. Table 4 shows the model coefficient and their significances estimated by multiples linear regression for carbofuran degradation time. By applying multiple regression analysis with neglecting the non-significant values, the simplified quadratic model was in the form of the following equation. The result showing the p-value <0.05 and a lack of fit that is not significant (0.2329) shows that the model is a very good predictor of the response variable. This is in agreement with the work of Mustapha *et al.* (2020) which found a significant model and not significant lack of fit shows good predictive capability of the reponse variable.

Coded equation of CCD for SAB2

Y = 0.493033 + -0.0008125 * A + 0.00102917 * B + 0.0154458 * C + 0.0303292 * D + -0.0263812 * AB + 0.00166875 * AC + 0.0204688 * AD + -0.00211875 * BC + 0.00683125 * BD + -0.0391437 * CD + 0.028276 * A^2 + -0.00812396 * B^2 + -0.013924 * C^2 + 0.00625104 * D^2 (4)

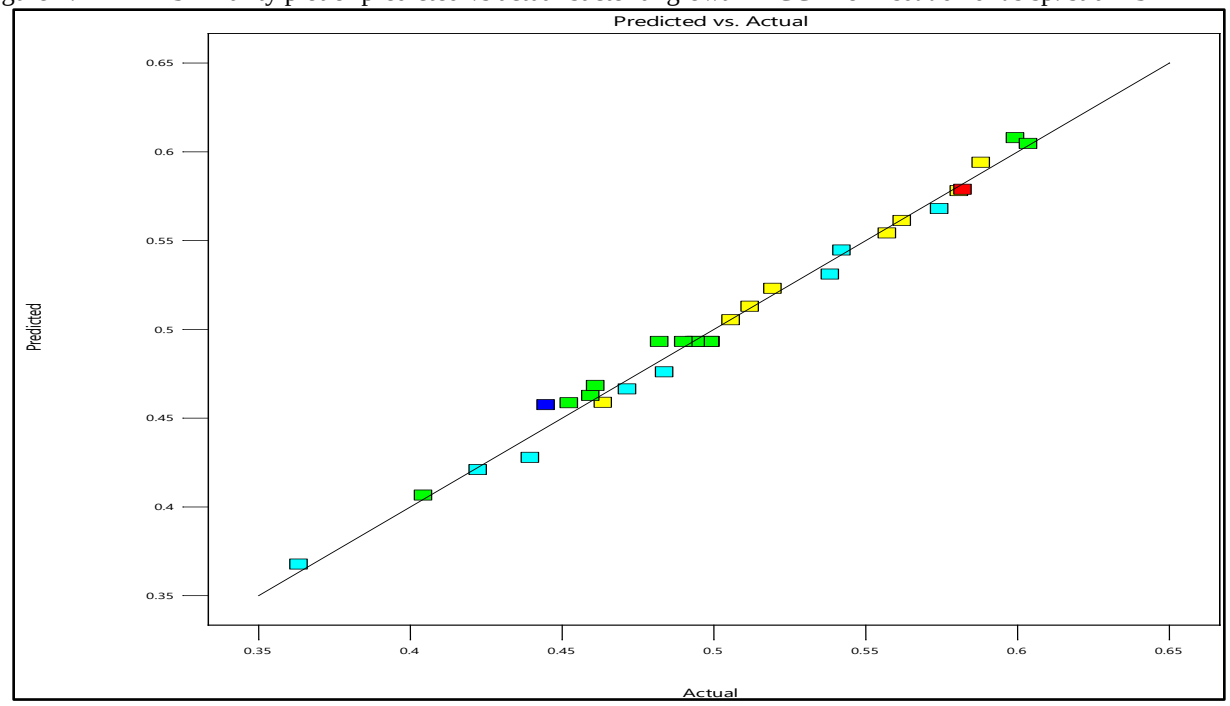
NB; A= Temperature, B= pH, C= Inoculum size, D = Carbon source

Actual equation of CCD for SAB2

Y = 2.5261699074075 + -0.18704351851852 * A + 0.39945833333333 * B + 0.44615833333332 * C + -0.91205000000002 * Carbon source + -0.0087937499999999 * A * pH + 0.0011125000000003 * A * C + 0.027291666666667 * A * D + -0.0042374999999995 * B * C + 0.027325000000001 * B * D + -0.31315 * C * D + 0.0031417824074074 * A^2 + -0.0081239583333334 * B^2 + -0.055695833333333 * C^2 + 0.10001666666667 * D^2 (5)

Figure 2 shows the similarity plot of Predicted vs Actual bacterial growth of strain SAB2. The data suggest that since both the predicted and experimental values have a significantly high degree of proximity to each other the model can be considered to be highly reliable and suitable model for the prediction of response variable. The R^2 values of 0.9907 for SAB2 or bacterial growth indicate a strong correlation between the actual and predicted responses.

Figure 2: Similarity plot of predicted vs actual bacterial growth in CCD for *Pseudomonas* sp. strain SAB2



The three-dimensional (3D) plots in Figure 3 (A - F) visually illustrate the relationship between bacterial growth and the different independent variables that affect the degradation capability of isolate SAB2 respectively. These plots depict similar trends, showing how bacterial growth varies with different combinations of process factors such as Temperature, pH, inoculum size and Carbon source. The plots illustrated the interactive effects of variables on for *Pseudomonas* sp. strain SAB2. The interaction between temperature and pH showed a pronounced synergistic effect, with maximum degradation observed within a moderately neutral pH range and mesophilic temperature conditions. This may be attributed to optimal enzyme activity and membrane transport efficiency under these environmental conditions. This is similar to the work of Mustapha *et al.* (2020) which also found that maximum degradation was observed neutral pH and mesophilic temperature. Similarly, interaction between inoculum size and carbofuran concentration indicated that an adequate biomass concentration was required to counteract potential substrate toxicity and enhance biodegradation efficiency.

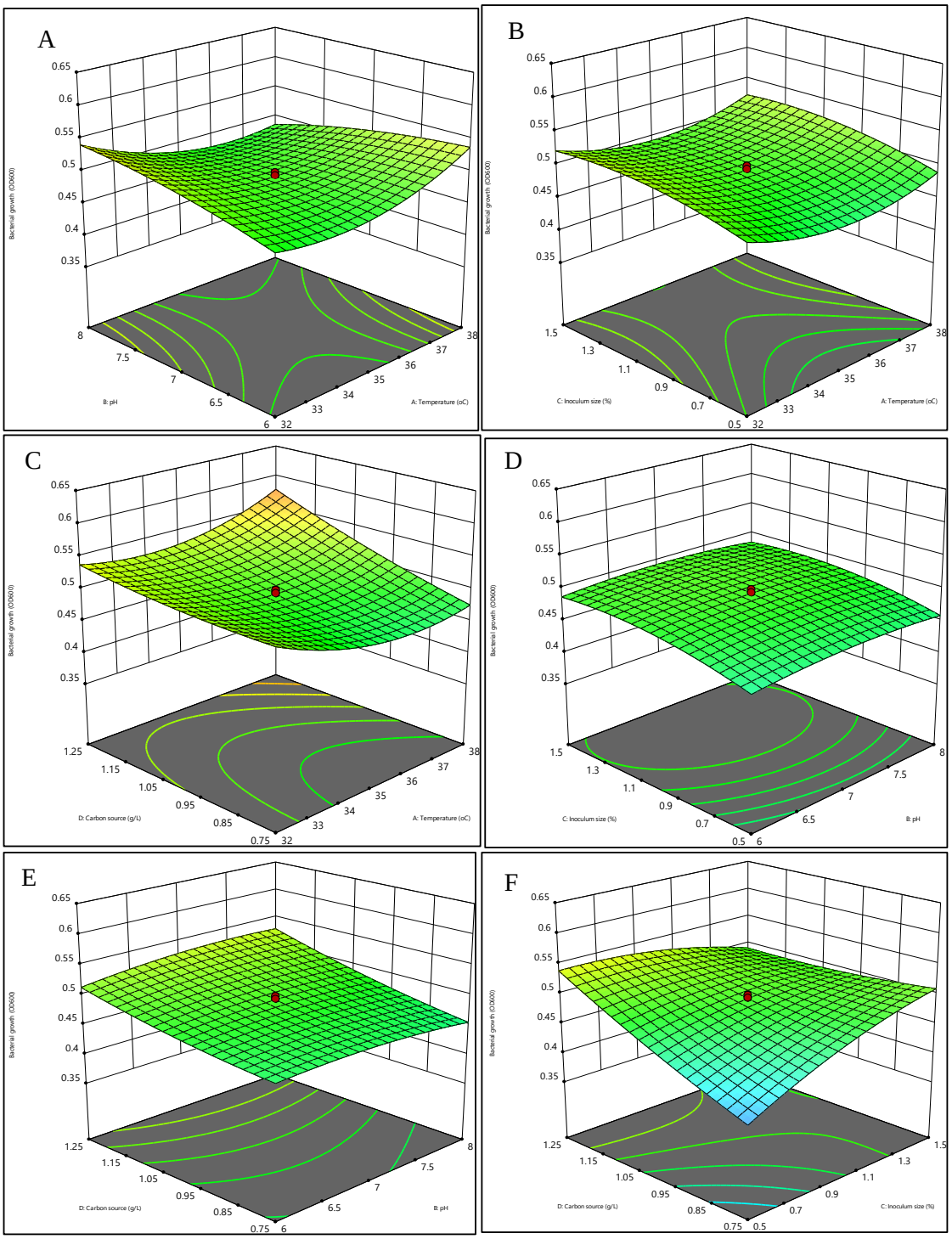


Figure 3: 3D plot of CCD for isolate SAB2

A: 3D plot showing the relationship between temperature and pH in CCD for Strain SAB2 B: 3D plot showing the relationship between temperature and inoculum size in CCD for Strain SAB2. C: 3D plot showing the relationship between temperature and carbon source in CCD for Strain SAB2. D: 3D plot showing the relationship between inoculum size and pH in CCD for Strain SAB2. E: 3D plot showing the relationship between Carbon source and pH in CCD

Validation of experiment

The optimized conditions predicted by the RSM model resulted in a maximum carbofuran degradation efficiency of strain SAB2 exceeding that observed during preliminary experiments. Validation experiments conducted under predicted optimal conditions closely matched the model's predicted value, confirming the robustness and predictive capability of the statistical model. The model predicted temperature (35°C), pH (7), inoculum size (1%) and carbon source (1g/L) while keeping the remaining factors constant. An experiment was conducted under optimal conditions as predicted by RSM for verification. T-test result showed that there was no significant difference between the predicted mean Bacterial growth (M = 0.4930) and the experimented bacterial growth mean (M = 0.5295) which indicates that experimentation is confirmed to be valid.

The enhancement in degradation efficiency under optimized conditions highlights the importance of parameter interaction in microbial bioprocesses. Compared to the conventional one-factor-at-a-time approach, RSM significantly improved process performance while reducing experimental runs and operational cost.

The bacterial isolate SAB2 was found to be a good candidate for carbofuran degradation and can potentially be effective in the degradation of other carbamate pesticides. Members of the genus *Pseudomonas* have been found to possess carbofuran hydrolase enzymes that break it down into less toxic intermediates. The degradation efficiency of the isolate was found to be maximized by fine tuning the physicochemical parameters affecting the growth of the isolate. The degradation efficiency obtained in this study compares favorably with previously reported carbofuran-degrading strains e.g. *Enterobacter cloacae* (Fareed *et al*., 2017), *Enterobacter* sp *(Mustapha et al.*, 2020) and *Pseudomonas aeruginosa* strain S07 (Patowary *et al*., 2023) suggesting that strain SAB2 has promising potential for bioremediation applications.

CONCLUSION

This study optimized carbofuran degradation by *Pseudomonas* sp. strain SAB2 using Response Surface Methodology. Plackett-Burman Design identified temperature, pH, inoculum size, and carbon source as significant factors ($P<0.05$), while Central Composite Design generated a statistically significant predictive model ($p<0.05$, $R^2=0.9907$). Degradation efficiency was markedly enhanced under optimized conditions achieving the highest bacterial growth OD600nm of 0.60 which shows the effectiveness of the CCD model in increasing the bacterial growth of the isolate. Experimental validation confirmed model reliability by showing that predicted mean Bacterial growth (M = 0.4930) and the experimented bacterial growth mean (M = 0.5295) were not statistically significantly different. *Pseudomonas* sp strain SAB2 can be potentially used for effective remediation of carbofuran contaminated sites.

Acknowledgement

We acknowledge Tertiary Education Trust Fund (Tetfund), Department of Biochemistry and Centre for Biotechnology Research, Bayero University Kano for providing an enabling the environment required to conduct the research.

REFERENCE

Ariffin, F., & Rahman, S. A. (2020). *Biodegradation of Carbofuran; A Review*. *8*(1), 50–57. https://doi.org/10.54987/jemat.v8i1.523

Babandi, A., U, L., S, E., Ibrahim, S., Anosike, C. A., & Yakasai, H. (2021). STATISTICAL OPTIMIZATION OF ALKALOIDS EXTRACTION FROM Ficus sycomorus LEAVES USING RESPONSE SURFACE METHODOLOGY. *Malaysian Society for Biochemistry & Molecular Biology*, *24*(3), 1–14.

Bas, D., Ismail, H., & Boyaci, J. (2007). Modeling and optimization I: usability of response surface methodology. *Journal of Food Engineering*, *78*(3), 836–845.

Bello, A. U., Mashi, J. A., Bababgana, K., Atiku, U. M., Yakubu, K., Babandi, A., & Ibrahim, S. (2026). A REVIEW ON CARBOFURAN IN THE ENVIRONMENT: PERSISTENCE, TOXICOLOGICAL IMPACTS AND MICROBIAL BIOREMEDIATION STRATEGIES. *FUDMA JOURNAL OF SCIENCES*, *10*(2), 131–144. https://doi.org/10.33003/fjs-2026-1002-4485

Fareed, A., Zaffar, H., Rashid, A., Maroof Shah, M., & Naqvi, T. A. (2017). Biodegradation of N-methylated carbamates by free and immobilized cells of newly isolated strain Enterobacter cloacae strain TA7. *Bioremediation Journal*, *21*(3–4), 119–127. https://doi.org/10.1080/10889868.2017.1404964

Ibrahim, S., Shukor, M. Y., Khalil, K. A., Halmi, M. I. E., Syed, M. A., & Ahmad, S. A. (2015). Application of response surface methodology for optimising caffeine-degrading parameters by Leifsonia sp. strain SIU. *Journal of Environmental Biology*, *36*(5), 1215–1221.

Ibrahim, S., Shukor, M. Y., Syed, M. A., Johari, W. L. W., Shamaan, N. A., Sabullah, M. K., & Ahmad, S. A. (2016). Enhanced caffeine degradation by immobilised cells of Leifsonia sp. strain SIU. *Journal of General and*

Applied Microbiology, *62*(1), 18–24. https://doi.org/10.2323/jgam.62.18

Ibrahim, S., Shukor, M. Y., Syed, M. A., Wan Johari, W. L., & Ahmad, S. A. (2016). Characterisation and growth kinetics studies of caffeine-degrading bacterium Leifsonia sp. strain SIU. *Annals of Microbiology*, *66*(1), 289–298. https://doi.org/10.1007/s13213-015-1108-z

Ibrahim, S., Zahri, K. N. M., Convey, P., Khalil, K. A., Gomez-Fuentes, C., Zulkarnain, A., Alias, S. A., González-Rocha, G., & Ahmad, S. A. (2020). Optimisation of biodegradation conditions for waste canola oil by cold-adapted Rhodococcus sp. AQ5-07 from Antarctica. *Electronic Journal of Biotechnology*, *48*, 1–12. https://doi.org/10.1016/j.ejbt.2020.07.005

Kaur, P., & Balomajumder, C. (2020). Bioremediation process optimization and effective reclamation of mixed carbamate-contaminated soil by newly isolated Acremonium sp. *Chemosphere*, *249*, 125982. https://doi.org/10.1016/J.CHEMOSPHERE.2020.125982

Lukman, K., Ibrahim, S., Muhammad, A., Babandi, A., Yakasai, H. M., Muhammad, J. B., & Jagaba, A. H. (2024). Bacillus sp. KS38 strain for sustainable caffeine degradation: Isolation, identification and optimization using response surface methodology. *Desalination and Water Treatment*, *320*(July), 100628. https://doi.org/10.1016/j.dwt.2024.100628

Mishra, S., Zhang, W., Lin, Z., Pang, S., Huang, Y., Bhatt, P., & Chen, S. (2020). Carbofuran toxicity and its microbial degradation in contaminated environments. *Chemosphere*. https://www.sciencedirect.com/science/article/pii/S0045653520316131

Mustapha, M. U., Halimoon, N., Johari, W. L. W., & Yunus, M. A. S. (2020). Optimization of carbofuran insecticide degradation by Enterobacter sp. using response surface methodology (RSM). *Journal of King Saud University - Science*, *32*(3), 2254–2262. https://doi.org/10.1016/j.jksus.2020.03.002

Patowary, R., Jain, P., Malakar, C., & Devi, A. (2023). *Biodegradation of carbofuran by Pseudomonas aeruginosa S07: biosurfactant production, plant growth promotion, and metal tolerance*. *30*(54), 115185–115198. https://link.springer.com/article/10.1007/s11356-023-30466-z

Plackett, R. ., & Burman, P., J. (1946). The Design of Optimum Multifactorial Experiments. *Biometrika*, *33*(4), 305. https://doi.org/10.2307/2332195

Randika, J. L. P. C., Bandara, P. K. G. S. S., Soysa, H. S. M., Ruwandeepika, H. A. D., & Gunatilake, S. K. (2022). Bioremediation of Pesticide-contaminated Soil: A Review on Indispensable Role of Soil Bacteria. *Journal of Agricultural Sciences - Sri Lanka*, *17*(1), 19–43. https://doi.org/10.4038/JAS.V17I1.9609

S. Ibrahim, A. Zulkharnain, K.N.M. Zahri1, G.L.Y. Lee, P. Convey, C. Gomez-Fuentes, S. Sabri, K.A. Khalil, S.A. Alias, G. G.-R. and S. A. A. (2020). Efecto de metales pesados y otros xenobioticos en la biodegradación de residuos de aceite de canola mediante la cepa adaptada al frío Rhodococcus sp. AQ5-07. *Revista Mexicana de Ingeniería Química*, *19*(3), 1041–1052. http://www.redalyc.org/articulo.oa?id=62029966013

Zhang, Y., Xu, Z., Chen, Z., & Wang, G. (2020). Simultaneous degradation of triazophos, methamidophos and carbofuran pesticides in wastewater using an Enterobacter bacterial bioreactor and analysis of toxicity and biosafety. *Chemosphere*, *261*. https://doi.org/10.1016/j.chemosphere.2020.128054