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Response Surface Methodology Optimization and Kinetics of Diesel Degradation by a Cold-Adapted Antarctic Bacterium, *Arthrobacter* sp. Strain AQ5-05

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Abstract: Petroleum hydrocarbons, notably diesel oil, are the main energy source for running amenities in the Antarctic region and are the major cause of pollution in this area. Diesel oil spills are one of the major challenges facing management of the Antarctic environment. Bioremediation using bacteria can be an effective and eco-friendly approach for their remediation. However, since the introduction of non-native organisms, including microorganisms, into the Antarctic or between the distinct biogeographical regions within the continent is not permitted under the Antarctic Treaty, it is crucial to discover native oil-degrading, psychrotolerant microorganisms that can be used in diesel bioremediation. The primary aim of the current study is to optimize the conditions for growth and diesel degradation activity of an Antarctic local bacterium, *Arthrobacter* sp. strain AQ5-05, using the Plackett-Burman approach and response surface method (RSM) via a central composite design (CCD) approach. Based on this approach, temperature, pH, and salinity were calculated to be optimum at 16.30 °C, pH 7.67 and 1.12% (*w/v*), respectively. A second order polynomial regression model very accurately represented the experimental figures' interpretation. These optimized environmental conditions increased diesel degradation from 34.5% (at 10 °C, pH 7.00 and 1.00% (*w/v*) salinity) to 56.4%. Further investigation of the kinetics of diesel reduction by strain AQ5-05 revealed that the Teissier model had the lowest RMSE and AICC values. The calculated values for the Teissier constants of maximal growth rate, half-saturation rate constant for the maximal growth, and half inhibition constants (μ_{max}, Ks, and Ki), were 0.999 h^{-1}, 1.971% (*v/v*) and 1.764% (*v/v*), respectively. The data obtained therefore confirmed the potential application of this cold-tolerant strain in the bioremediation of diesel-contaminated Antarctic soils at low temperature.

Keywords: Antarctica; *Arthrobacter*; diesel bioremediation; growth kinetics; response surface methodology

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1. Introduction

Human activity in Antarctica, particularly the establishment and operation of research stations and various forms of hydrocarbon-powered transport, brings with it the threat of hydrocarbon spills, notably diesel oil leaking from storage tanks, and spills in the transportation of fuel as well as the refueling of generators and vehicles [1–3]. Diesel oil spills are the main cause of pollution caused directly by human activities on the Antarctic continent and potentially create serious impacts on native ecosystems [4]. Diesel oil, the common widely used oil in Antarctica, is made up of aliphatic and aromatic hydrocarbons that are persistent if released into the environment [5,6], due to the frequently low temperatures, nutrient inaccessibility and arid conditions, which inhibit the rate of bacterial processes and abiotic degradation [7,8].

The development of microbial bioremediation procedures with the potential of removing environmental contaminants can offer a safe and cost-effective alternative to disposing of contaminants or contaminated soils in waste dump sites or the use of physico-chemical approaches [9,10], and has been suggested as a suitable technique for remediating contaminated polar soils [11,12]. However, in Antarctica, the application of native Antarctic microbes for such engagements is mandatory as the use and release of foreign organisms, including transferring between different recognized biogeographic regions within Antarctica [13], is not allowed under the Ecological Etiquette to the Antarctic Agreement [14]. A number of researchers have demonstrated the competence of local microorganisms to degrade petroleum hydrocarbon pollutants in the unfriendly environmental conditions of Antarctica [7,15–17].

Similar to other xenobiotics, bacterial growth on this toxic substrate shows an important lag phase owing to the needs of the cells to initiate decontamination by degradation using enzymes upon contact with diesel oil before assimilation can occur. The growth profile displays several phases: the specific growth rate is initially zero, followed by a slow rate related to lag time (λ); this is followed by an increase to a maximum value (μ_{max}) which is sustained for a period of time; finally, growth enters a decay phase where the rate reduces and eventually reaches zero or an asymptote (A). The maximum growth rate (μ_{max}) is the most important parameter of growth [18]. This value is important for secondary models including growth kinetics. In many studies, this parameter has been assessed manually by subjectively defining the portion of the growth curve that is almost linear and then determining the slope of this section by linear regression. An improved approach is to describe the entire dataset using a nonlinear regression growth model and then to estimate μ_{max}, λ, and A from the model [19]. Furthermore, many earlier studies did not attempt any further fitting of the data to available models [18]. The sigmoidal growth curve can be described by various mathematical functions including Logistic, Gompertz, Richards or Schnute [18], Baranyi-Roberts [20], Von Bertalanffy [21], Buchanan three-phase [22] and, more recently, Huang models [23]. Besides providing predictive ability and internal reliability, the value of a model is also influenced by its mathematical ease, manageability, the number of its adjustable parameters and, where appropriate, whether or not it has intuitive meaning [24].

The utilization of hydrocarbons by microorganisms has been described to be strongly dependent on environmental parameters including pH, temperature, nitrogen sources, and salinity [25,26]. Consequently, these environmental parameters are key features influencing the success of diesel biodegradation. Optimization of the conditions for microbial diesel degradation is crucial to guarantee effective treatment.

The one-factor-at-a-time (OFAT) approach is a traditional method for optimizing diesel oil degradation [27]. However, this technique is laborious, time consuming, and eliminates interactions between different variables, resulting in data misinterpretation. These drawbacks can be avoided using statistical approaches of optimization including the Plackett-Burman design (PBD) and Response Surface Methodology (RSM) [28]. PBD has the practical advantage of decreasing the number of experiments required by offering a means of identifying the most relevant parameters from a broad range available. RSM, via the application of central composite design (CCD), is utilized to study the

influence of different factors that affect responses by varying them simultaneously and performing a number of experiments [29]. RSM is an assemblage of experimental methods, statistical interventions, and mathematical approaches for exploring and developing an estimated suitable correlation between a response parameter and a set of design parameters.

Arthrobacter sp. strain AQ5-05, a cold-tolerant bacterium and phenol degrader from Antarctic soil [30], was assessed here for its potential to degrade diesel oil as a solely carbon source. This bacterium is responsive to environmental and medium factors including temperature, pH, nitrogen source, and salinity [30]. Thus, optimizing these parameters is essential in order to achieve maximum diesel oil degradation. The present study set out to optimize diesel degrading parameters and study the growth kinetics of diesel reduction by this bacterial strain in order to determine its potential to bioremediate diesel oil-contaminated sites in cold areas.

2. Materials and Methods

2.1. Samples and Media

Arthrobacter sp. strain AQ5-05 used in this research was formerly isolated from soil collected from King George Island, South Shetland Islands, maritime Antarctic [30]. Diesel oil used for the research described here was acquired from a Petronas filling station in Selangor, Malaysia, and was utilized as the only carbon source in all testing. Bushnell-Haas (BH) broth medium was employed for the screening and optimization of growth studies [31]. The medium used consist of (g/L): $CaCl_2$ (0.02), $MgSO_4$ (0.2), NH_4NO_3 (1.0), $FeCl_3$ (0.05), and K_2HPO_4 (1.0). The pH of the BH medium was amended to 7.0 ± 0.2 at 25 °C before sterilization by 15-min autoclaving at 121 °C. To determine the capability of the strain to utilize diesel fuel as the lone carbon source, an uninoculated BH broth was used as control. All experiments were carried in triplicate.

2.2. Screening for Diesel-Degrading Competence

Strain AQ5-05 was pre-cultured in a 250 mL flask in nutrient broth at 10 °C for 24 h on an incubator shaker at 150 rpm. Following incubation, the growth culture was centrifuged for 10 min at 5000 rpm and the resulting bacterial pellets were rinsed with 1× phosphate-buffered saline, and then re-suspended to an OD_{600} of 1 in BH broth comprising 0.5% (*v/v*) diesel oil as the only carbon source. The diesel medium was incubated on a shaking incubator at 150 rpm at 10 °C for 168 h. The incubation temperature of 10 °C was picked to mimic a characteristic soil surface temperature in summer on King George Island and more widely in the maritime Antarctic [32,33]. The competence of strain AQ5-05 to degrade diesel oil was monitored at 24 h intervals, while colony forming units (cfu/mL) were used to determine bacterial growth. The remaining hydrocarbon in diesel from the culture medium was removed every 12 h by a solvent removal process by destroying sampling of triplicate flasks consisting in growth culture medium (50 mL of BH broth in 250 mL conical flask); n-hexane to media (1:1) was utilized to remove the cellular material. Following evaporation, the remaining hydrocarbon was measured using the gravimetric method [34]. Percentage diesel degradation was evaluated using Equation (1).

$$\text{Diesel degradation } (\%) = \frac{a-b}{a} \times 100 \tag{1}$$

where:

a = initial mass of diesel

b = mass of remaining diesel fuel in experiment sample.

Following 7 d culture, the volume of diesel degraded was analyzed in the extracted diesel oil test samples. Additionally, to check the degradation capability of AQ5-05, both the solvent (n-hexane)-extracted diesel oil treated samples and inert control were analyzed using a gas chromatography-mass spectrometer (GC/MS-QP2010 Ultra, Shimadzu, Japan, fitted through an automatic injector GC2010) [35]. The GC (30 m × 0.25 mm × 0.25 μm) platform was optimized and all

experiments were conducted thru a 10:1 riven ratio. The carrier gas (helium) has a movement speed of 0.8 mL min^{-1}, and was maintained at 250 °C injection temperature. The temperature of the column oven was programmed at 50 °C for 10 min and was afterwards raised to 300 °C with a gradient of 8 °C min^{-1} and lastly held for 37 min. The electron ionization mode (70 eV) was used for receiving mass spectrometric data. The temperatures of both the ion source as well as the MS interface were programmed at 200 and 250 °C, independently. The mass range (m/z) was designated as 40–700 for the whole analysis.

2.3. Experimental Design and Data Analysis

2.3.1. Plackett-Burman Design (PBD)

A competent method to detect relevant factors among a huge number of variables [36] was employed to screen relevant variables that considerably affect diesel degradation. Here, a 12-run PBD was used to evaluate five independent variables; i.e., temperature, ammonium sulfate concentration, pH, salinity, and substrate concentration. Each independent variable had two points: −1 (low level) and +1 (high level). The two level values were set based on initial experimental trials (data not shown). The PBD and the response values of diesel degradation are presented in Table 1.

Table 1. Placket-Burman experimental design matrix with growth of strain AQ5-05.

Run	A	B	C	D	E	Growth (Log Cfu)
1	6.0	5.0	8.0	0.6	0.0	6.93
2	6.0	25.0	6.5	0.6	0.0	7.08
3	6.0	5.0	6.5	0.2	3.0	7.22
4	6.0	25.0	6.5	0.6	3.0	7.35
5	1.5	25.0	8.0	0.2	3.0	7.33
6	1.5	25.0	6.5	0.2	0.0	7.37
7	1.5	5.0	8.0	0.6	3.0	7.03
8	1.5	25.0	8.0	0.6	0.0	7.27
9	1.5	5.0	6.5	0.2	0.0	6.97
10	1.5	5.0	6.5	0.6	3.0	7.08
11	6.0	5.0	8.0	0.2	0.0	6.87
12	6.0	25.0	8.0	0.2	3.0	7.09

A: diesel concentration; B: temperature; C: pH; D: $(NH_4)_2SO_4$; E: NaCl.

All tests were performed in triplicate and the mean of the percentage degradation was utilized as the response variable, Y. A second-order model was employed to define the interaction within the independent variables and the response. The second-degree polynomial model was:

$$
\begin{aligned}
Y = \beta_0 + \beta_1 X_1 + \quad & \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{11} X_1 + \beta_{22} X_2 + \beta_{33} X_3 + \beta_{44} X_4 \\
& + \beta_{55} X_5 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{15} X_1 X_5 + \beta_{23} X_2 X_3 \\
& + \beta_{24} X_2 X_4 + \beta_{25} X_2 X_5 + \beta_{34} X_3 X_4 + \beta_{35} X_3 X_5 + \beta_{45} X_4 X_5
\end{aligned}
\tag{2}
$$

where Y is the expected response factor; and β_0, β_1, β_2, β_3, β_4, β_{11}, β_{22}, β_{33}, β_{44}, β_{55}, β_{12}, β_{13}, β_{23}, β_{24}, β_{14}, and β_{15} are constant regression factors of the model, in which β_0 is the intercept term, β_{11}, β_{22}, β_{33}, β_{44}, and β_{55} are the squared constants, β_{12}, β_{13}, β_{23}, β_{24}, β_{14}, and β_{15} are the relations constants and β_1, β_2, β_3, β_4, and β_5 are the linear coefficients. X_1, X_2, X_3, and X_4 are the independent factors. Parameter permutations (X_1, X_2) display the relationship between the variables.

2.3.2. Response Surface Methodology

RSM involves using a cluster of arithmetical and statistical methods to define the correlation between the independent parameters and the response [37]. The optimal levels of relevant variables ($p < 0.05$) screened in PB were chosen and optimized in a centre composite design (CDD) [38]. In this study, a three-factor, five-level CCD with 20 runs was utilized. The tested variables included

temperature, pH, and salinity, and were represented as B, C, and E respectively, and each was assessed at five different levels (−2, −1, 0, 1, 2).

The relationship between response (diesel) and independent factors was described using the CCD quadratic model based on the second-order polynomial equation:

$$Y = \beta \sum_{i=1}^{K} \beta_i X_i + \sum_{i=1}^{\kappa} \beta_{ii} \chi_i^2 + \sum_{1 \le i \le j}^{\kappa} \beta_{ij} \chi_i \chi_j \tag{3}$$

where Y is the diesel degradation (response), X_i and X_j are independent factors, k is the integer of factors, β_0 is the model intercept, β_I is the linear constant, and β_{ii} is the quadratic constant [39]. The significance of the model and regression coefficient were determined using analysis of variance (ANOVA). The coefficient (R^2) was determined to assess the fit of the model, and Fisher's F-test was used to evaluate the statistical relevance. The response surface and contour structures of the model-expected responses were used to evaluate the mutual corrections between the relevant parameters.

2.3.3. Validation of Model

The RSM-generated mathematical model was validated by performing an experiment using the predicted optimum medium conditions.

2.4. Determination of Growth Kinetic Parameters

Validation of inherent growth kinetic variables can be achieved by modeling built on various substrate impeding models, including the Luong, Yano, Teissier–Edward, Aiba, Haldane, Monod, and Han–Levenspiel models, in contrast to the frequently applied Monod model, owing to the recognized hindrance consequence of high diesel concentration to bacterial growth [40–42]. The formulae for these models are presented in Table 2. Plotting bacterial numbers against time allowed the desirability of the precise growth rate constant, μ, at individual original diesel concentration to be acquired. Upon plotting these values against substrate concentration, a non-linear curve is obtained, with modeling used to determine the constant.

Table 2. Mathematical models used in growth kinetics studies.

Model	No. of Parameters	Equation	Reference (s)
Monod	2	$\mu_{max} \frac{s}{k_s + s}$	[43]
Haldane	3	$\mu_{max} \frac{s}{s + k_s + \frac{s^2}{k_i}}$	[44]
Teissier	3	$\mu_{max}\left[1 - \exp\left(\frac{s}{k_i}\right) - \exp\left(\frac{s}{k_i}\right)\right]$	[45]
Aiba	4	$\mu_{max} \frac{s}{K_s + S} \exp\left(-\frac{S}{K_i}\right)$	[46]
Yano and Koga	4	$\frac{\mu_{max} S}{s + k_s + \left(\frac{s^2}{k_1}\right)\left(1 + \frac{s}{k}\right)}$	[47]
Luong	4	$\frac{\mu_{max} S}{k_s + S}\left(1 - \frac{s}{s_m}\right)^n$	[48]
Han and Levenspiel	5	$\frac{\mu_{max}\left(1 - \frac{s}{s_m}\right)^n}{k_s + S - \left(1 - \frac{S}{S_m}\right)^m}$	[49]

μ_{max} represents the maximum specific growth rate (h^{-1}), where K_s is the half saturation constant (g/L), K_i denotes the self-inhibition constant (g/L), K_m signifies the critical inhibition concentration (g/L), S is the substrate inhibition, S_m is the critical substrate concentration, and n is the exponent signifying the impact of the substrate on μ_{max}.

2.5. Data Analyses

Design-Expert Version 6.0.8 (Stat-Ease Inc., Minneapolis, MN, USA) was employed for experimental design, and for regression analyses, and pictorial presentation of the experimental data acquired. Seven various kinetic models were built to the experimental figures via CurveExpert Professional Software (Version 1.6) with custom equation procedure utilized to detect the coefficients.

3. Results

3.1. Screening of Relevant Parameters Using PBD

The response illustrated in Table 1 revealed a significant difference in growth (cfu/mL) of strain AQ5-05 in just 12 runs. AQ5-05 grew exponentially ranging from 6.87 to 7.21 cfu/mL, indicating that the studied parameters effectively enhanced bacterial growth. The greatest growth was observed in run no. 6 (25 °C, pH 6.5, NaCl at 0.20 g/L). The significant model terms were B, C, E, AB, AE, BD, and BE (Table 3), confirming that the factors that significantly influenced the strain's growth were temperature, pH, and salinity. The effects of ammonium sulfate concentration and substrate concentration were not significant and these were therefore excluded from the subsequent optimization experiment using CCD. The several regression analyses of the responses give rise to the quadric equation:

$$Y = +7.13 +0.054A + 0.12B + 0.062C - 8.60D +0.11E - 0.025AB^2 +0.044AE^2 + 8.600BC^2 + 0.058BD^2 + 0.030BE^2 \tag{4}$$

Table 3. Analysis of variance (ANOVA) for diesel degradation by strain AQ5-05.

Source	Sum of Squares	DF	Mean Square	*F*-Value	Prob > *F*
Model	0.32	10	0.04	538.94	0.034 *
A	0.03	1	0.03 3.66	144.39	0.053
B	0.15	1	0.15	597.90	0.008 *
C	0.03	1	0.03	999.53	0.020 *
D	4.93	1	4.93	19.45	0.142
E	0.09	1	0.09	355.35	0.011 *
AB^2	4.38	1	4.38	172.70	0.048 *
AE^2	0.01	1	0.01	499.92	0.0285 *
BC^2	4.93	1	4.93	19.45	0.142
BD^2	0.02	1	0.02	895.39	0.021 *
BE^2	6.37	1	6.37	251.41	0.040 *
Residual	6.00	1	6.00		
Cor total	0.32	11			
Standard deviation	7.746			R^2	0.999
Mean	7.13			Adjusted R^2	0.998
C.V	0.11			Predicted R^2	0.913
PRESS	0.028			Adequate R^2	66.611

A: diesel concentration; B: temperature; C: pH, D: $(NH_4)_2SO_4$; E: NaCl; *: $p < 0.05$.

The model suitability was checked using analysis of variance (ANOVA), giving a significant outcome ($F = 424.23$, $p = 0.0335$).

3.2. Optimization by Response Surface Methodology

3.2.1. RSM Regression Equation and Model Analysis

The interaction between the relevant parameters was further studied with CCD and their optimum levels were determined. Table 4 shows the experimental matrix of CCD giving the actual and predicted values for diesel degradation by strain AQ5-05. The highest diesel degradation occurred in run no. 20 (56.32%), compared to the predicted value of 63.40%, while the lowest occurred in run no. 9 (4.30%) (Figure 1). Multiple regression analysis was used to create the second-order polynomial equation:

$$Y = +56.32 + 3.38A - 1.28B - 1.68C - 19.68A^2 - 16.51B^2 - 1.98C^2 - 1.25AB + 0.95AC + 0.70BC \tag{5}$$

Analyses of variance confirmed that the model was fit for prediction contained by the range of the employed variables (Table 5).

Table 4. Experimental central composite design (CCD) data of diesel degradation by strain AQ5-05.

Run	A	B	C	Diesel Degradation (%)	
				Predicted Value	**Experimental Value**
1	5.0	6.50	0.0	13.0	18.14
2	15.0	7.25	4.02	26.10	25.49
3	25.0	8.0	3.0	10.90	16.67
4	31.82	7.25	1.5	15.20	19.02
5	15.0	8.51	1.5	8.60	11.48
6	15.0	7.25	−1.02	21.70	22.63
7	5.0	8.0	3.0	5.50	12.81
8	15.0	7.25	1.5	17.40	18.96
9	−1.82	7.25	1.5	−5.01	4.30
10	25.0	6.5	3.0	6.50	6.34
11	15.0	7.25	1.5	13.50	11.77
12	15.0	7.25	1.5	15.20	7.40
13	15.0	7.25	1.5	58.70	53.54
14	5.0	8.0	0.0	52.20	47.89
15	25.0	8.0	0.0	58.60	46.32
16	25.0	6.50	0.0	50.00	52.32
17	15.0	5.99	1.5	56.50	46.32
18	15.0	7.25	1.5	54.30	51.32
19	5.0	6.5	3.0	53.50	50.32
20	15.0	7.25	1.5	63.40	56.32

A: temperature; B: pH; C: salinity.
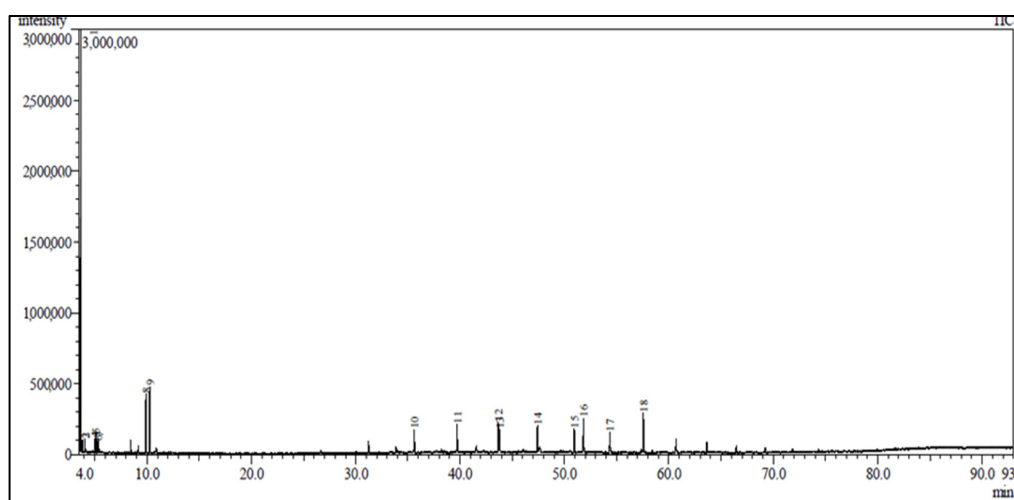
Figure 1. Gas Chromatography-Mass Spectrometer (GCMS) profile of diesel oil (3% (*v/v*)) extracted from aqueous phase of the medium after 7 days of incubation with strain AQ5-05.

3.2.2. Interaction between the Relevant Parameters

Figure 2 presents the graphical illustrations of the regression model, termed response surface plots, along with their comparable contour plots that were obtained using Design-Expert software. Individual plots show the effects of dual independent parameters, keeping the other variables at zero level. The contour plot shape suggests whether the independent parameters interact perfectly. The interaction between temperature (A) and pH (B) on diesel reduction is illustrated in Figure 2A. The highest diesel degradation (56.45%) was observed between pH 7.25 and 7.68 and temperature 15 to 20 °C. In the interaction between temperature (A) and salinity (C) (Figure 2B), the highest diesel degradation was attained with a salinity range of 0.75% to 1.50% (*w/v*) and temperature of 15 to 20 °C. Figure 2C illustrates the interaction between pH (B) and salinity (C), where the highest diesel degradation took place between pH 7.25 and 7.63 and salinity 0.0% to 0.75% (*w/v*).

Table 5. Analysis of variance (ANOVA) of diesel mineralization by strain AQ5-05.

Source	Sum of Square	DF	Mean Square	*F*-Value	Prob > *F*
Model	893.83	9	992.20	22.46	< 0.001 *
A	155.61	1	155.61	3.52	0.090
B	22.53	1	22.53	0.51	0.492
C	38.51	1	38.51	0.87	0.373
A^2	558.04	1	558.04	126.30	< 0.001 *
B^2	393.70	1	393.70	88.94	< 0.001 *
C^2	56.61	1	56.61	1.28	0.284
AB	12.50	1	12.50	0.28	0.606
AC	7.22	1	7.22	0.16	0.695
BC	3.92	1	3.92	0.09	0.772
Residual	441.86	10	44.19		
Lack of fit	334.96	5	66.99	3.13	0.118
Pure error	106.90	5	21.38		
Cor total	9371	19			
Std. dev.	6.65		R^2	0.953	
Mean	30.26		Adjusted R^2	0.910	
C.V	21.97		Predicted R^2	0.712	
PRESS	2698.58		Adeq Precision	13.049	

A: temperature; B: pH; C: salinity; *: $p < 0.001$.

(**A**)　　　　　(**B**)　　　　　(**C**)
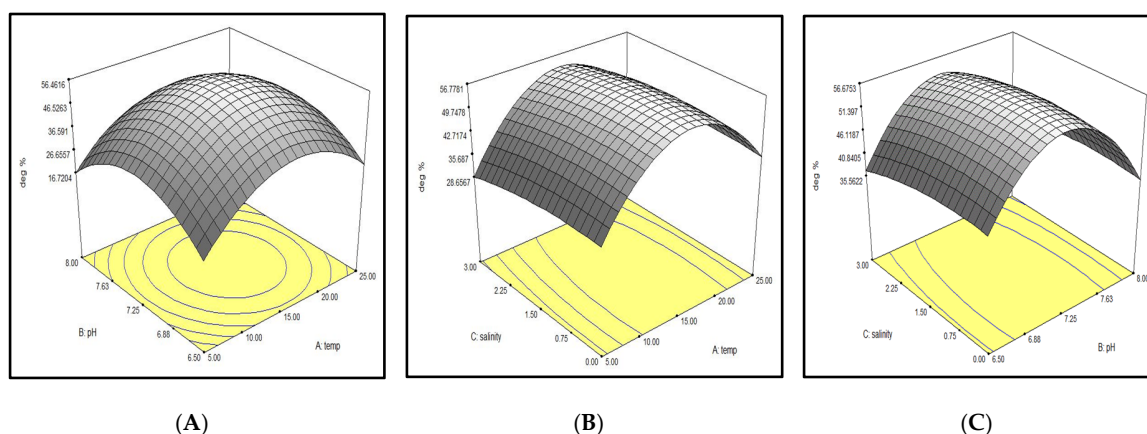
Figure 2. The interactions between (**A**) temperature and pH, (**B**) temperature and salinity, and (**C**) pH and salinity, illustrating their effect on diesel degradation.

3.2.3. Model Validation

The model was validated by performing a test experiment using conditions of 3% (*v/v*) diesel, 15 °C, pH 7.25 and salinity 1.5% (*w/v*), as predicted by RSM. Under these optimized conditions, 56.32% diesel degradation was predicted. The experimental diesel degradation under these conditions was also 56.32%, further supporting the validity of the model.

3.3. Growth Kinetics Studies

In this study, the modified Gompertz model was utilized to model the influences of diesel on the initial activity of the enzyme (Figure 3). Strain AQ5-05 was able to grow on 2% (*v/v*), 3% (*v/v*) and 3.5% (*v/v*) diesel oil, with decreasing growth with increasing initial diesel concentration.

The microbial growth curves and equation derived from the Gompertz model were used to explain the log (exponential) and stagnant (stationary) phases of the sigmoidal bacterial growth curves. The specific growth rate of strain AQ5-05 was fitted to seven kinetic models of growth, namely Luong, Yano, Tessier-Edward, Aiba, Haldane, Mono, and Han-Levenspiel (Figure 4). All the models gave a good fit of specific growth rate except for the Monod model.
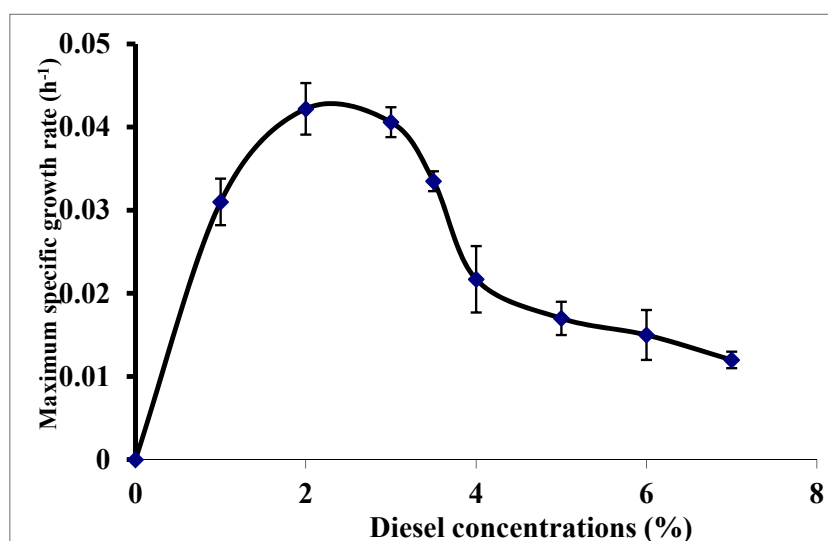
Figure 3. Effect of diesel concentration on the growth of strain AQ5-05 in culture at 10 °C. Bacterial growth was measured by determination of colony-forming units (log CFU/mL).
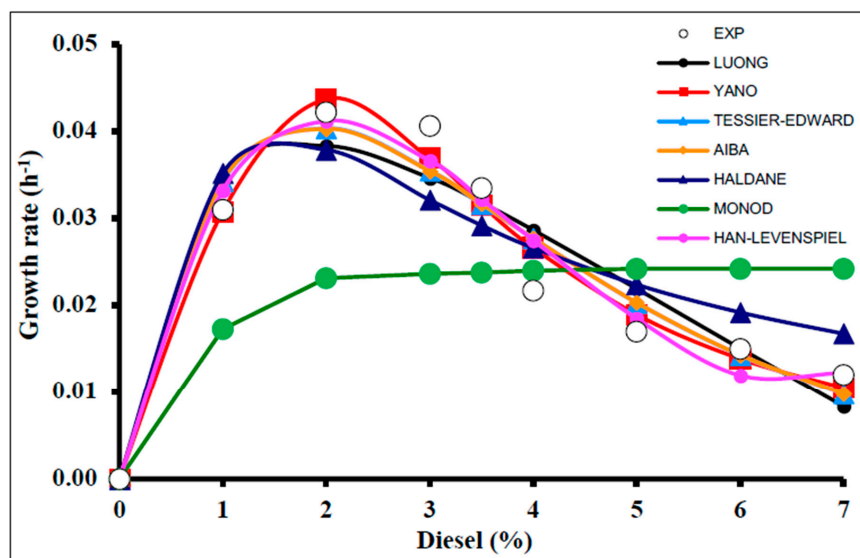

Figure 4. Curve fitting of experimental growth rate for *Arthrobacter* sp. strain AQ5-05 using various kinetics models.

Comparing the models, the most suitable model for strain AQ5-05 was Teissier, which exhibited low AICc and RMSE values, the highest adjusted R^2 value, and an F test along with an accuracy factor and bias factor nearest to unity (1.0) (Table 6). The suitability for the linear regression model was assessed using the R^2, while for the nonlinear regression the adjusted R^2 was utilized [50].

Table 6. Statistical analysis of growth kinetics models for *Arthrobacter* sp. strain AQ5-05.

Model	Parameter	RMSE	R^2	ad R^2	AIC$_C$	BF	AF
Luong	4	0.00555	0.892	0.784	−61	0.0997	1.158
Yano	4	0.0032	0.927	0.934	−71	0.996	1.084
Tessier-Edward	3	0.0040	0.934	0.895	−79	1.003	1.120
Aiba	3	0.0040	0.933	0.895	−79	1.004	1.120
Haldane	3	0.0061	0.794	0.671	−72	1.0081	1.203
Monod	2	0.0132	−1.399	−2.199	−65	0.953	1.509
Han and Levenspiel	5	0.0041	0.8786	0.8786	−42	1.001	1.509

The calculated values for the Tessier coefficients of maximal growth rate, half-saturation rate coefficients for the maximal growth, and half inhibition coefficients, denoted by μ_{max}, K_s, and K_i, were 0.999 h^{-1}, 1.971% (*v/v*), and 1.764% (*v/v*), respectively. It should be noted that the μ_{max} value attained from curve-fitting interpolation is not the true value, as the true μ_{max} is where the incline for the slope is zero. Hence, the value was approximately 0.0422 h^{-1} at 2% (*v/v*). The equation generated by the Tessier model using the values obtained was:

$$0.0422\left[1 - \exp\left(\frac{s}{1.971_i}\right) - \exp\left(\frac{s}{1.764}\right)\right] \tag{6}$$

The correlation constant for the Tessier substrate disruption model was 0.934, while the Aiba and Yano models gave similar values of 0.933 and 0.949, respectively.

4. Discussion

Antarctica is an isolated and arid region. Its persistently low temperatures are a problem for bacterial activity. Yet, human engagements in the southern polar region have been linked with the occurrence of contamination actions. These activities are unavoidably linked with the usage of diesel oil and, consequently, the incidence of inadvertent oil leaks [16,51]. The cold-tolerant bacterial strain *Arthrobacter* sp. AQ5-05, originally isolated from Antarctic soil, was tested and confirmed for its ability to utilize diesel as its lone source of carbon and energy. Under our experimental protocol, the strain attained huge diesel degradation within seven days following an early acclimatization period of 24 h. Previously, species of *Bacillus, Enterobacter, Pseudomonas, Rhodococcus,* and *Arthrobacter* have been described as being capable of diesel degradation [30,52,53] and there are also reports of petroleum hydrocarbons degrading *Arthrobacter* sp. strains (n-alkanes and phenol) at lower temperature, similar to the results obtained in this study.

Application of PBD was used to identify variables significantly influencing diesel degradation. The results obtained confirmed that nitrogen source ((NH_4)$_2SO_4$) and substrate concentration did not significantly influence degradation by *Arthrobacter* sp. AQ5-05. However, temperature, pH, and salinity influenced degradation significantly. These finding concur with the reports of other research [8,30] on Antarctic bacteria. In addition, the determination coefficient (R^2) of 0.9998 is an indication that the predicted and the experimental values were in good agreement.

Highest diesel degradation (56.45%) achieved using PBD occurred at pH 7.25–7.68 and 15–20 °C, the latter being consistent with the psychrotolerant nature of strain AQ5-05 [30]. Several other diesel oil degrading microbes have been described to exhibit maximum degradation at near-neutral pH [8,54]. In addition, the oval nature of the 3D surface plot also suggests a mutual interaction between these variables, with the centre of the structure signifying the point where maximum diesel degradation was attained [55].

The CCD further optimized the relevant parameters identified in PBD. Modeling kinetics showed that each of the models tested, excluding the Monod model, described the experimental results well. The Teissier model proved to provide the best overall adequate fit to the experimental data. The Teissier approach was designed to model substrate-limited growth kinetics by diffusing the substrate above the external membrane of the bacterium [45]. The current findings are similar to the reduction of diesel by *Burkholderia* sp. strain DRY27 [56] and in biodegrading oil and lubricant in effluent using activated sludge [57]. However, a considerable difference was apparent in terms of the determent effect of diesel oil between strain DRY27 and AQ5-05. In the former, growth was almost completely inhibited at 3.215% (*v/v*) diesel. In the latter, growth was not inhibited at concentrations of diesel of 3–5% (*v/v*).

5. Conclusions

This study has demonstrated that the cold-tolerant diesel-degrading *Arthrobacter* strain AQ5-05 from Antarctica is capable of degrading up to 3% (*v/v*) diesel at 10 °C within seven days. Optimizing the environmental variables influencing diesel degrading activity via RSM greatly enhanced degradation.

Between 10 and 15 °C the strain attained maximum diesel degradation, proving that the polar summer soil environs offers suitable environmental variables for potential diesel removal by this strain. The application of mathematical growth models revealed important growth constants, which are useful in estimating diesel utilization and the diesel concentration that may be significant during upscaling of any diesel bioremediation process. Studies are ongoing to identify the pathways used for diesel degradation using a whole genome sequencing approach, which will support the further improvement and application of this bacterium in the area of bioremediation techniques.

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