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Statistical optimisation for enhancement of phenol biodegradation by the Antarctic soil bacterium *Arthrobacter* sp. strain AQ5-15 using response surface methodology

K. Subramaniam[1], N.A. Shaharuddin[1], T.A. Tengku-Mazuki[1], A. Zulkharnain[2], K.A. Khalil[3], P. Convey[4] and S.A. Ahmad[1,5]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[2]Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama-337 8570, Japan
[3]Department of Biomolecular Sciences, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor, Malaysia
[4]British Antarctic Survey, NERC, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom
[5]National Antarctic Research Centre, B303 Level 3, Block B, IPS Building, Universiti Malaya, 50603, Kuala Lumpur, Malaysia

***Corresponding Author Email :** aqlima@upm.edu.my

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Abstract

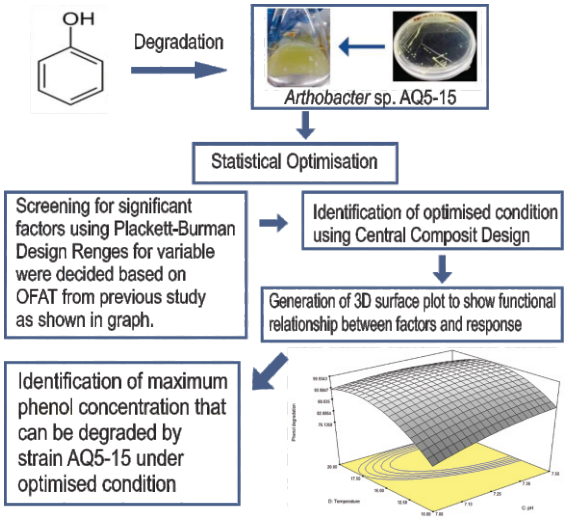
Aim: Effective bioremediation requires optimisation of conditions under which the process takes place. In this study, an Antarctic soil bacterium, *Arthrobacter* sp. strain AQ5-15, was evaluated for phenol biodegradation under statistically optimised conditions.

Methodology: The composition of degradation media and the culture conditions for this study were determined according to the experimental requirements obtained from Plackett-Burman factorial design (PB) and Box-Wilson Central Composite Design (CCD), respectively. Phenol degradation was monitored by 4-aminoantipyrine colorimetric assay and bacterial growth was quantified by measuring optical density ($OD_{600\,nm}$) at 72 hr.

Results: A preliminary screening experiment using the Plackett-Burman design indicated that all the factors screened (ammonium sulphate concentration, sodium chloride concentration, pH and temperature) had significant influence on degradation performance. Response Surface Methodology was then utilised to further optimise the phenol-degrading process using Central Composite Design. The maximum percentage of phenol degradation achieved with CCD was 99.42%, under medium conditions of 0.15 g l^{-1} $(NH_4)_2SO_4$, 0.13 g l^{-1} NaCl, pH 7.25 and incubation at 15°C for 72 hr. The strain could degrade phenol when exposed to an initial concentration of up to 1.5 g l^{-1} under these optimised conditions.

Interpretation: The tolerance and degradation characteristics of strain AQ5-15 suggest that it has potential application in bioremediation of polluted sites and in the treatment of relatively cool water bodies contaminated with phenol.

Key words: *Arthrobacter* sp., Aromatic hydrocarbon, Bioremediation, Indigenous, Psychrotolerant



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Introduction

Phenol is an aromatic hydrocarbon commonly derived from industrial activities. It has been classified as a high priority toxicant by the United States Environmental Protection Agency (US EPA, 2014) due to its toxicity to living systems and recalcitrant nature. Since Industrial Revolution, there has been a progressive increase in pollution across the globe (Téllez-Pérez *et al.,* 2013). Among pollutants, phenol contamination has become one of the most prevalent in the environment, requiring urgent mitigation actions as it may cause negative impacts on biological systems and also human beings (Zhou *et al.,* 2011; Lee *et al.,* 2017). Anthropogenic pollution is apparent even in the continent that many consider to be the most pristine and isolated, Antarctica. Since the mid-Twentieth Century, the construction and operation of over 50 Antarctic research stations, increasing tourism activities, increased shipping and air transportation and the rapid growth of industrial activities in distant countries of Southern and Northern Hemispheres have resulted in detectable pollution, including phenol, in Antarctica (Bargagli, 2008; Mazuki *et al.,* 2019). Antarctica's ecosystems and biota are highly sensitive to pollution. Furthermore, the continent's chronically cold and harsh climate means that the natural processes involved in remediation of pollution occurs slowly as compared to others places. As a result, pollutants can bioaccumulate in Antarctica's simple ecosystems and food chains (Jara-Carrasco *et al.,* 2017).

Phenol can be removed from the environment by physical and chemical means (Mijangos *et al.,* 2006; Caetano *et al.,* 2009; Mohammadi *et al.,* 2015). However, biological remediation technology has become the preferred approach due to their cost effectiveness, ecofriendly nature, applicability, and usually generates non-toxic end products (Ahmad *et al.,* 2017; Suárez-García *et al.,* 2019). The Antarctic Treaty prohibits the importation of non-native organisms into Antarctica, meaning that application of bioremediation approaches in this continent can only be achieved using indigenous microorganisms (Ibrahim *et al.,* 2020). A number of studies have reported the ability of native Antarctic microorganisms, mainly from the genera *Arthrobacter* and *Rhodococcus,* to degrade phenol at low temperature (Ahmad *et al.,* 2018; Zakaria *et al.,* 2018). However, identifying such microbes is the stepping stone for the development of bioremediation process. Environmental factors and nutritional parameters such as temperature, pH, salinity, and the presence and type of nitrogen and carbon sources exert important influence on the phenol-degrading capacity and growth rate of microorganisms (Lee *et al.,* 2018; Tengku-Mazuki *et al.,* 2020). Consequently, it is important to optimise these factors to enhance the bioremediation process at laboratory scale, before making an attempt to apply the process in the field environment.

Conventionally, a method such as one-factor-at-time (OFAT) is used for this optimisation process, allowing assessment of optimum value of each factor individually (Vera Candioti *et al.,* 2014). However, this approach is time-consuming and expensive in the case of involvement of multiple variables. In addition, it may not predict the true optimal conditions since interactions between the factors are neglected (Vajić *et al.,* 2015). With advances in statistical analyses and information technology, numerous statistical software packages have been developed as optimisation tools to aid in bioreactor design (Nawawi *et al.,* 2016; Yusuf *et al.,* 2016; Yaacob *et al.*, 2016). One of the well-known software, Design-Expert Version 6, is programmed to calculate the statistical values and probability to define the minimum run of experiments required to recognize significant cause-and-effect relationships between a given number of variables and responses.

This software comprise four categories of Design of Experiment (DoE) which are factorial, response surface, mixture and crossed-process mixture (Alben, 2002). Mathematical and statistical approaches such as Response Surface Methodology (RSM) can be used in optimising multiple factors collectively, avoiding the limitations of single factor optimisation approaches such as OFAT (Zhou *et al.,* 2011). RSM is a sequential procedure that begins with significant factor screening using a two-level factorial design such as Plackett-Burman design (PB) to find the region of optimum, and then modelling and optimising the response with a three-level factorial design such as Box-Behnken Central Composite Design (CCD) (Ibrahim *et al.,* 2015; Karamba *et al.,* 2016). In recent years, many studies have reported the use of RSM to statistically optimise phenol-degrading processes (Sivasubramanian and Namasivayam, 2015; Priyadharshini and Bakthavatsalam, 2016). However, as yet, there has been no application of statistical optimisation for phenol degradation by bacterial strains indigenous to Antarctic continent. The current study addresses statistical approach optimisation using PB design and CCD to enhance the phenol-degrading process by native bacterium, *Arthrobacter* sp. strain AQ5-15.

Materials and Methods

Inoculum preparation: *Arthrobacter* sp. strain AQ5-15,a soil bacterium previously isolated from the western part of King George Island, Antarctica (Subramaniam *et al.,* 2019) was used in this experiment. The bacterial strain was retrieved from storage in glycerol at -80°C, grown and maintained in nutrient broth (Friendemann Schmidt, Australia) at 10°C on an orbital shaker (Protech Model 722, Malaysia) agitated at 150 rpm, in the Eco-Remediation Technology Laboratory, Universiti Putra Malaysia. About 10% (v/v) of culture was used as standard inoculum throughout this optimisation study.

Optimisation study: Minimal salt medium (MSM) containing 0.5 g l^{-1} phenol was inoculated with 10% (v/v) of bacterial culture and incubated on the shaking incubator at 150 rpm for 72 hr. Uninoculated sterile MSM was used as control for confirming the ability of strain to utilise phenol as a sole carbon source. MSM comprised (per litre) K_2HPO_4 (0.4), KH_2PO_4 (0.2), NaCl (0.1), Mg_2SO_4, (0.1), $(NH_4)_2SO_4$ (0.4), $MnSO_4.H_2O$ (0.01), $Fe_2(SO_4).H_2O$ (0.1) and $NaMoO_4.H_2O$ (0.1). However, the composition of

degradation media and the culture conditions for this study were determined according to the experimental requirements obtained from Plackett-Burman factorial design (PB) and Box-Wilson Central Composite Design (CCD), respectively. Phenol degradation was monitored at 72 hr using 4-aminoantipyrine colorimetric assay following the of American Public Health Association (APHA, 2005). Based on the method, the pH of phenol media to be tested was adjusted to pH 10, followed by addition of one percent of 4-aminoantipyrine and potassium ferricyanide from the total media to be tested. The change in colour of the solution and bacterial growth (OD_{600}) were quantified using a UV/Visible spectrophotometer (Jenway Model 7305, UK) at 510 nm and 600 nm, wavelength, respectively.

Experimental design: Researchers focus on statistical approaches like RSM that often produce enormous return-of-investment (ROI) at this breakthrough phase (Anderson and Whitcomb, 2004). The core idea of RSM is to use a sequence of designed experiments to achieve one or more optimal responses. The experimental design and statistical analyses were generated using Design-Expert Version 6.0.8 (Stat-Ease Inc. Minneapolis, USA). Each variable ($(NH_4)_2SO_4$ concentration, NaCl concentration, pH and temperature) was screened using PB, followed by CCD to identify the optimum levels of significant variables obtained from optimisation via OFAT approach from previous study, characterising the response surface in the selected experimental region. Both experiments were run in triplicate with the average of phenol degradation percentage and bacterial growth taken as the responses. The influential variables ($p<0.05$) screened by PB were selected and optimised using quadratic factorial CCD by combining two factorial points, a sole central point and two axial points (+2, +1, 0, -1, -2) (Table 1) leading to a total of 30 runs. The response from the interactions between different factors was specified using a second-order polynomial regression model comprising linear, quadratic and interaction coefficients, as given in eq. 1:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_{i2} + \sum_{1<i<j}^{k} \beta_{ij} x_i x_j \quad (1)$$

where, y represents the response variable, x_i are the independent variables that affect *y*, *k* is the number of variables, β_0 indicates the intercept term, β_i is the i^{th} linear coefficient, β_{ii} is the i^{th} quadratic coefficient and β_{ij} is the ij^{th} interaction coefficient; *i* and *j* = 1, 2, 3 where $i \neq j$ are coefficients in the model. In constructing the regression equation for application, the experimental variables were coded based on eq. 2:

$$x_i = \frac{(x_i - x_f)}{\Delta x_i} \quad (2)$$

where, X_i is the coded value of i^{th} independent variable, x_i is the uncoded value of i^{th} independent variable, x_f is the uncoded value of i^{th} independent variable at the centre point and Δx_i is the step change value. The codes are calculated as a function of range of interest of each variable as shown in Table 2. The significance of model and each coefficient in the equation were examined using Fischer's F-test and Analysis of Variance (ANOVA). The response surface and 3D contour plots of the predicted model were used to evaluate the interaction between significant variables.

Results and Discussion

As a first step, Plackett Burman factorial design (PB) was used as it is an effective screening method to identify most important variables among multiple variables that have a positive influence on the process being examined (Patil and Jena, 2015; Priyadharshini and Bakthavatsalam, 2016). The significance of four factors, namely $(NH_4)_2SO_4$ concentration (x_1), NaCl concentration (x_2), pH (x_3) and temperature (x_4) in phenol degradation by AQ5-15 were analysed with 12 different combinations of experiments as shown in Table 3. The range for all four variables affecting phenol degradation was decided based on the result from OFAT as follows: 0.4 - 0.6 g l^{-1} $(NH_4)_2SO_4$, 0.1 - 0.15 g l^{-1} NaCl, pH of 7.0 - 7.5, temperature at 10°C-20°C (Subramaniam *et al.,* 2019). The initial concentration of 0.5 g l^{-1} was selected for optimisation based on previous studies which used this concentration for screening for phenol biodegradation (Lee *et al*., 2018) as higher phenol concentration can be inhibitory to the growth of psychrotolerant bacteria (Li *et al.,* 2010; Sepehr *et al.,* 2019). Table 3 shows both experimental and predicted values for phenol degradation by strain AQ5-15, based on the 12 runs given by PB design. Fischer's F-test showed that the model was significant ($F = 558.8259$, $p = 0.0018$) (Table 4).

All four variables exhibited significant effects on phenol degradation and, hence, were employed in the designing of CCD. Das and Chandran (2011) noted that nutrient availability, especially nitrogen source, can act as a limiting factor, and that nutrient concentration might either enhance or inhibit

Table 1: Experimental ranges of independent variables for optimisation using CCD expressed as actual and coded levels

Variable	Symbol	Coded level				
		-2	-1	0	1	2
$(NH_4)_2SO_4$ concentration (g l^{-1})	x_1	0.3	0.4	0.5	0.6	0.7
NaCl concentration (g l^{-1})	x_2	0.08	0.1	0.13	0.15	0.17
pH	x_3	6.75	7.00	7.25	7.50	7.75
Temperature (°C)	x_4	5	10	15	20	25

Table 2: Relationship between coded and actual value of factors

Coded value	Actual value
-2	^{x}min
-1	$[\frac{(^{x}max+^{x}min)}{2}]-[\frac{(^{x}max-^{x}min)}{2\beta}]$
0	$[\frac{(^{x}max+^{x}min)}{2}]$
1	$[\frac{(^{x}max+^{x}min)}{2}]-[\frac{(^{x}max-^{x}min)}{2\beta}]-$
2	^{x}max

β is $2^{n/4}$; n is the number of variables where n=4

degradation. NaCl concentration can adversely affect the biological performance of microorganisms dramatically, for instance affecting the suspended solid concentration in effluent and, thus, reducing the efficiency of organic compound removal by inhibiting bacterial metabolism (Chen *et al.,* 2018). pH is also considered highly significant because most microbes are unable to tolerate high or low pH (Shah, 2018). Finally, temperature can affect both the physical state of pollutants as well as directly influence microbial growth rates and metabolic process (Varjani and Upasani, 2017; Subramaniam *et al.,* 2019).

The analysis of variance (ANOVA) of experimental results from the PB design showed that the model was well supported (Table 4). The F-value of 12.637 implied that Lack of Fit was not significant relative to the pure error where there was a 17.46% chance that a "Lack of Fit F-value" this large could occur due to noise. Insignificancy of lack of fit is good for the model to be fit (Sanusi *et al.,* 2016). ANOVA on the model design displayed a R^2 of 0.9996 showing the closeness of data to the fitted regression line. The "Pred R-Squared" of 0.9442 was in reasonable agreement with the "Adj R-Squared" of 0.9978 and the "Adeq Precision" of 60.396 indicated an adequate and satisfactory signal. A low coefficient of variation of value (0.43%) showed that the model was precise and highly reliable. The CCD design of RSM experiments, using all four significant variables from PB, with the experimental and predicted values for phenol degradation are presented in Table 5. A total of 30 sets of experiments were generated by CCD using different sets of values for the variables, including outliers. The maximum percentage of phenol degradation was observed in run 16 (99.42%) with medium conditions of 0.15 g l^{-1} $(NH_4)_2SO_4$, 0.13 g l^{-1} NaCl, pH 7.25 and incubation temperature of 15°C. The regression equation coefficients were calculated as shown in Table 6, and the data were fitted to a second-order polynomial equation to be used for predicting optimum combination of conditions. The relationship between phenol degradation and the variables is shown in eq. (3) and (4) in terms of actual and coded factors, respectively. The predicted response is indicated by Y and x_1, x_2, x_3 and x_4 are the coded values of $(NH_4)_2SO_4$, NaCl, pH and temperature, respectively. Positive and negative signs in the regression equation signify either synergistic or antagonistic effects of each variable on the response.

$$Y = -2027.03896 + 88.62292x_1 + 482.02500x_2 + 501.75500x_3 + 27.02208x_4 - 88.46875x1^2 - 1883.50000x2^2 - 31.61500x3^2 - 0.34849x4^2 - 2.11950x_3x_4 \quad (3)$$

$$Y = + 98.37 + 0.015x_1 + 0.28x_2 + 2.89x_3 + 6.01x_4 - 0.88x1^2 - 1.18x2^2 - 1.98x3^2 - 8.71x4^2 - 2.65x_3x_4 \quad (4)$$

The ANOVA of experimental results for CCD showed that the experimental data fitted well to the statistical model, with a statistically significant model (Table 6). The "Lack of Fit F-value" of 3.50 indicated 8.86% chance that a "Lack of Fit F-value" this large could occur due to noise and insignificant lack of fit is needed for a model to fit. The value of Prob>F<0.0001 illustrated that the two linear terms (x_3, x_4), all four quadratic terms ($x1^2$, $x2^2$, $x3^2$, $x4^2$) and one interaction term (x_3x_4) of the model were significant, and the model specified that the two most influential variables were pH (x_3) and temperature (x_4). Based on the regression analysis of the model, the coefficient determination, R^2 was found to be 0.9825 presenting a high correlation between the

Table 3: Experimental design given by PB and results on phenol degradation by strain AQ5-15 using different parameters

Run	x_1	x_2	x_3	x_4	Experimental value (%)	Predicted value (%)
1	0.60	0.10	7.50	20.00	92.25	92.36
2	0.40	0.15	7.50	20.00	92.00	91.77
3	0.40	0.10	7.00	10.00	76.30	76.07
4	0.40	0.15	7.00	10.00	71.89	72.00
5	0.60	0.10	7.50	10.00	82.76	82.53
6	0.40	0.10	7.00	20.00	89.87	89.98
7	0.40	0.15	7.50	10.00	85.68	85.79
8	0.40	0.10	7.50	20.00	88.68	88.79
9	0.60	0.10	7.00	10.00	72.15	72.26
10	0.60	0.15	7.00	20.00	91.95	91.79
11	0.60	0.15	7.50	10.00	91.23	91.34
12	0.60	0.15	7.00	20.00	91.75	91.79

Table 4: ANOVA for phenol degradation by strain AQ5-15 using PB design

Source	Sum of Squares	DF	Mean Square	F-Value	Prob > F
Model	685.8599	9	76.20665	558.8259	0.0018**
x1	16.95402	1	16.95402	124.3243	0.0079**
x2	10.44493	1	10.44493	76.59303	0.0128*
x3	140.8485	1	140.8485	1032.847	0.0010***
x4	261.7335	1	261.7335	1919.3	0.0005***
x1x2	15.79722	1	15.79722	115.8415	0.0085**
x1x3	6.732016	1	6.732016	49.36609	0.0197*
x1x4	6.892879	1	6.892879	50.54571	0.0192*
x2x3	23.05602	1	23.05602	169.0706	0.0059**
x3x4	29.21922	1	29.21922	214.2655	0.0046**
Residual	0.272738	2	0.136369		
Lack of Fit	0.252738	1	0.252738	12.63692	0.1746
Pure Error	0.02	1	0.02		
Cor Total	686.1326	11			
Std. Dev	0.37			R^2	0.9996
Mean	85.54			Adj R^2	0.9978
C.V.	0.43			Pred R^2	0.9442
PRESS	38.30			Adeq precision	60.396

*: p<0.05; **: p<0.01; ***: p<0.001

Table 5: Experimental design given by CCD and results on phenol degradation by strain AQ5-15 using significant parameters from PB

Run	x_1	x_2	x_3	x_4	Experimental value (%)	Predicted value (%)
1	0.50	0.08	7.25	15.00	94.14	93.10
2	0.70	0.13	7.25	15.00	94.48	94.86
3	0.40	0.15	7.50	10.00	85.68	85.35
4	0.50	0.17	7.25	15.00	94.98	94.21
5	0.60	0.15	7.00	10.00	75.28	74.90
6	0.40	0.10	7.00	20.00	90.87	91.62
7	0.50	0.13	6.75	15.00	86.45	84.69
8	0.30	0.13	7.25	15.00	96.98	94.80
9	0.40	0.10	7.50	10.00	85.39	85.90
10	0.40	0.15	7.00	20.00	87.60	90.12
11	0.50	0.13	7.25	5.00	50.25	51.51
12	0.50	0.13	7.25	15.00	98.36	98.37
13	0.60	0.10	7.50	20.00	92.37	91.53
14	0.50	0.13	7.25	15.00	96.23	98.37
15	0.60	0.15	7.50	10.00	87.96	85.97
16	0.50	0.13	7.25	15.00	99.42	98.37
17	0.40	0.15	7.00	10.00	72.07	74.28
18	0.60	0.15	7.50	20.00	91.23	93.20
19	0.40	0.15	7.50	20.00	92.04	90.60
20	0.60	0.10	7.50	10.00	82.76	83.36
21	0.40	0.10	7.00	10.00	76.29	74.83
22	0.50	0.13	7.25	25.00	78.59	75.53
23	0.50	0.13	7.75	15.00	96.28	96.23
24	0.50	0.13	7.25	15.00	98.45	98.37
25	0.40	0.10	7.50	20.00	88.68	92.10
26	0.60	0.10	7.00	10.00	72.15	72.29
27	0.50	0.13	7.25	15.00	98.32	98.37
28	0.60	0.10	7.00	20.00	90.29	91.06
29	0.50	0.13	7.25	15.00	99.41	98.37
30	0.60	0.15	7.00	20.00	91.95	92.73

Table 6: ANOVA for phenol degradation by strain AQ5-15 using CCD

Source	Sum of squares	DF	Mean square	F-Value	Prob > F
Model	3282.67	9	364.74	90.47	< 0.0001***
x_1	5.704E-003	1	5.704E-003	1.415E-003	0.9704
x_2	1.86	1	1.86	0.46	0.5042
x_3	199.93	1	199.93	49.59	< 0.0001***
x_4	865.56	1	865.56	214.70	< 0.0001***
$x1^2$	21.47	1	21.47	5.32	0.0318*
$x2^2$	38.01	1	38.01	9.43	0.0060**
$x3^2$	107.09	1	107.09	26.56	< 0.0001***
$x4_2$	2081.89	1	2081.89	516.40	< 0.0001***
x_3x_4	112.31	1	112.31	27.86	< 0.0001***
Residual	80.63	20	4.03		
Lack of Fit	73.86	15	4.92	3.64	0.0803
Pure Error	6.77	5	1.35		
Cor Total	3363.30	29			
Std. Dev	2.01			R^2	0.9760
Mean	88.17			Adj R^2	0.9652
C.V.	2.28			Pred R^2	0.9320
PRESS	228.76			Adeq precision	40.423

*: p<0.05; **: p<0.01; ***: p<0.001

Table 7: Model validation for phenol degradation by strain AQ5-15

Factors	Predicted value	Experimental value
$[(NH_4)_2SO_4]$ (g l^{-1})	0.50	0.50
[NaCl] (g l^{-1})	0.10	0.10
pH	7.04	7.04
Temperature (°C)	15.76	16.00
Phenol degradation (%)	95.33	96.42

experimental design data. The "Pred R-Squared" of 0.9209 is in reasonable agreement with the "Adj R-Squared" of 0.9682. "Adeq Precision" of 35.732 indicated an adequate signal as it measures that the signal to noise ratio and a ratio greater than 4 is desirable. These results specify that these significant factors can act as limiting factors and minute variations in their value can affect the degradation rate of phenol.

Similarity between the predicted and actual analyses of the effects of variables on phenol degradation is shown in Fig. 1. The plot displays a close correlation between predicted and actual data as data points were accumulated closer to the line that bisects the plot at an angle of 45°. Based on Table 6 and Fig. 1, it can be deduced that the predicted values attained from the quadratic model were in good agreement with the experimental values (Sanusi *et al.,* 2016). This RSM tool comprised features for point prediction and interpret graphically through 3D response surface curve that could be handy in predicting the optimum value of significant factor interaction for maximum phenol degradation efficiency. Fig. 2 illustrates the 3D plot for optimum phenol degradation with combination of significant variables (x_3 and x_4). The slightly elliptical 3D plot shows interaction between the two most significant factors namely pH and temperature. The curved response surface specified that there are well-defined optimal variables because flat surface near the optimum means the optimised values may not differ largely from single variable conditions (Ibrahim *et al.,* 2015). The model predicted that maximum phenol degradation will be achieved between pH 7.13 - 7.5 and temperature range of 15°C - 17.5°C. Here it can be observed that the efficiency of phenol degradation having a negative effect at low temperature and acidic condition as well as at high temperature and alkaline condition. The temperature effect was more notable at pH 7 and 7.5, thus maximum phenol degradation can be achieved at neutral or near neutral condition with increased temperature.

Temperature is normally considered as one of the most influential factors, particularly in polar region as degradation must obey the Arrhenius law. According to the Arrhenius relationship, the degradation of a pollutant is highly dependent on temperature in where an increase or decrease in temperature can affect the rate of degradation (Arrhenius, 1889; Kulkarni *et al.,* 2017). This

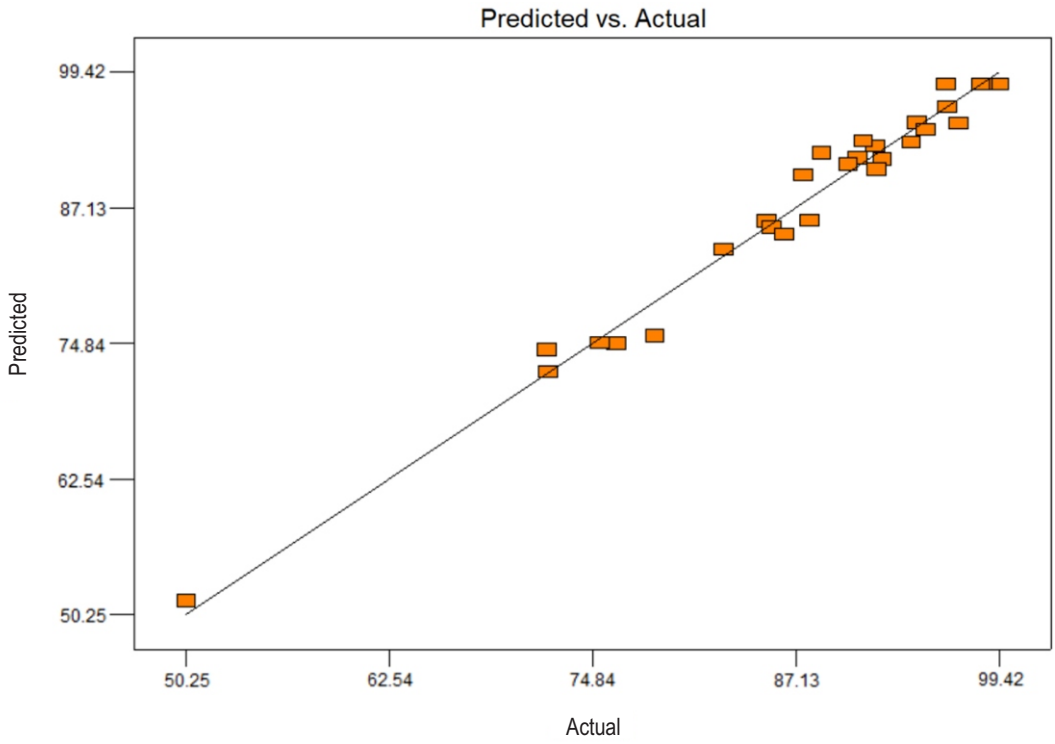

Fig. 1: Similarity plot between predicted and actual values for phenol biodegradation by strain AQ5-15.

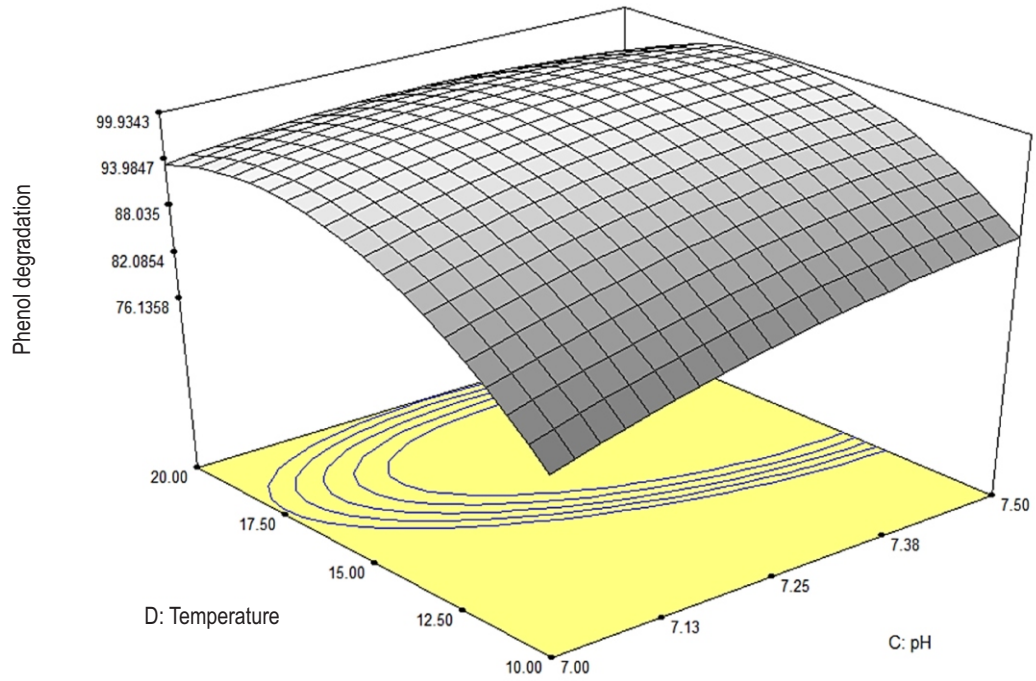

Fig. 2: 3D response surface plot showing interaction effect between significant variables, pH and temperature during phenol biodegradation by the strain AQ5-15.

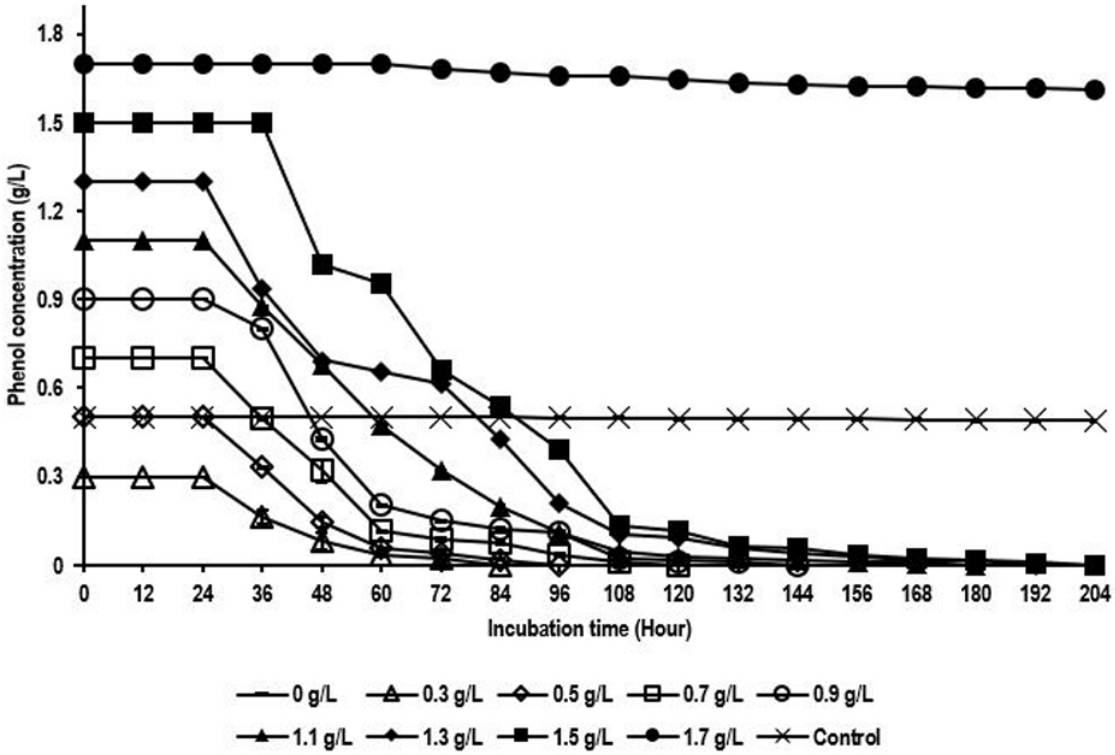

Fig. 3: Biodegradation of different initial concentrations of phenol by strain AQ5-15 under optimised conditions. Error bars represent mean ± standard deviation (n = 3).

optimum temperature for phenol degradation by strain AQ5-15 supports the potential use of this strain for phenol degradation in soils in cold regions. Similar to this strain, most hydrocarbon-degrading Antarctic bacteria have been reported to be facultatively psychrotolerant with optimum temperature for biodegradation activity ranging between 10-20°C (Gran-Scheuch *et al.,* 2017; Roslee *et al.,* 2019).

However, highly seasonal polar regions like Antarctica generally have annual mean air temperature below 0°C and reach positive daily values only for few short summer months (1-4 months) or weeks. Consistent with this, Convey *et al.* (2018) reported that on Signy Island, the annual mean of ground temperature is 1.7°C whereas annual maximum and minimum is 18.4 and -8.7°C, respectively. As for air temperature, the mean monthly temperature reach low positive values during summer, with annual mean of -3.9°C and annual maximum and minimum of 8.6 and -30.1°C, respectively. Biological activity will be minimal or undetectable during sub-zero habitual temperatures. Hence, any bioremediation approaches will only be effective during brief polar summer period when, with positive temperatures, unfrozen soils will have accessible water sources (Lee *et al.,* 2018). It is important to note that strain AQ5-15, being psychrotolerant where the strain is viable at temperature range of 5-25°C (Subramaniam *et al.,* 2019) and even at optimum temperature >15°C, is able to grow and function well at suboptimal positive temperatures, hence this strain still has the potential to grow and degrade phenol sub-optimally in Antarctic soils. The RSM model was validated using the values predicted for each parameter as shown in Table 7. The result showed no significant difference between the percentage of phenol degradation given by both predicted (95.33%) and experimental approaches (96.42%).

Fig. 3 shows degradation of different concentrations of phenol by strain AQ5-15 under optimised conditions obtained from CCD. The strain has the ability to degrade phenol up to 1.5 g l^{-1} within 24 hr. On increacsing the phenol concentration to 1.7 g l-1, the strain started to lose its degradation ability. Studies on cold tolerant bacteria isolated from alpine soils of Binaloud Mountains have shown that *Pseudomonas* sp. strain ATR208 can completely degrade phenol up to 0.6 g l^{-1} at higher time (Sepehr *et al.,* 2019). On the basis of previous studies, Antarctic microorganisms are reported to degrade phenol up to 0.5 g l^{-1} at temperature as low as 10°C (Gerginova *et al.,* 2013; Lee *et al.,* 2018). Phenol is a major constituent in fuel oil, pharmaceutical and personal care products (Kim *et al.,* 2018; Harley *et al.,* 2019) and can be released into the Antarctic environment via many channels, particularly during marine accidents and the disposal of 'grey water' wastes from research stations (Emnet *et al.,* 2015; Vázquez *et al.,* 2017; Martorell *et al.,* 2019). Singh *et al.* (2013)

reported that petroleum wastewater containing 140-480 mg l^{-1} of oil included phenol at concentrations ranging from 1.2-3.71 mg l^{-1}. Antarctica has also experienced multiple marine accidents with the release of large quantities of oil. For instance, a major fuel oil spill occurred in 1989 when the Argentine vessel *Bahia Paraiso,* that was *en route* to resupply an Argentinian research station, ran aground and sank near Palmer Station, Anvers Island, Antarctic Peninsula, releasing over 150 000 gallons of diesel (Kennicutt *et al.,* 1991). Recently, a similar event took place in the Galápagos Islands in December 2019, where a barge carrying 600 gallons of diesel oil sank off the dock (Oxford Analytica, 2019). Having highly persistent and anti-microbial properties, phenol can accumulate in the environment to high concentration (Krastanov *et al.,* 2013; Sachan *et al.,* 2019). Thus, this is the first report of an Antarctic bacterium *Arthrobacter* sp. strain AQ5-15 with higher tolerance and ability to degrade high concentration of phenol at 15°C.

In conclusion, statistical optimisation by RSM revealed that the combination of 0.5 g l^{-1} $(NH_4)_2SO_4$, 0.13 g l^{-1} NaCl, pH 7.25, and temperature of 15°C resulted in maximum phenol degradation by the native Antarctic soil bacterium, *Arthrobacter* sp. strain AQ5-15. The strain could tolerate and degrade an initial concentration of up to 1.5 g l^{-1} of phenol under optimised conditions. According to this study, the statistical approach could be a valuable alternative to the conventional one-factor-at-time (OFAT) method for the optimisation of phenol degradation in wastewater treatment.

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