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Optimisation of Various Physicochemical Variables Affecting Molybdenum Bioremediation Using Antarctic Bacterium, *Arthrobacter* sp. Strain AQ5-05

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Abstract: The versatility of a rare metal, molybdenum (Mo) in many industrial applications is one of the reasons why Mo is currently one of the growing environmental pollutants worldwide. Traces of inorganic contaminants, including Mo, have been discovered in Antarctica and are compromising the ecosystem. Bioremediation utilising bacteria to transform pollutants into a less toxic form is one of the approaches for solving Mo pollution. Mo reduction is a process of transforming sodium molybdate with an oxidation state of 6+ to Mo-blue, an inert version of the compound. Although there are a few Mo-reducing microbes that have been identified worldwide, only two studies were reported on the microbial reduction of Mo in Antarctica. Therefore, this study was done to assess the ability of Antarctic bacterium, *Arthrobacter* sp. strain AQ5-05, in reducing Mo. Optimisation of Mo reduction in Mo-supplemented media was carried out using one-factor-at-a-time (OFAT) and response surface methodology (RSM) approaches. Through OFAT, Mo was reduced optimally with substrate concentration of sucrose, ammonium sulphate, and molybdate at 1 g/L, 0.2 g/L, and 10 mM, respectively. The pH and salinity of the media were the best at 7.0 and 0.5 g/L, respectively, while the optimal temperature was at 10 °C. Further optimisation using RSM showed greater Mo-blue production in comparison to OFAT. The strain was able to stand high concentration of Mo and low temperature conditions, thus showing its potential in reducing Mo in Antarctica by employing conditions optimised by RSM.

Keywords: Antarctica; molybdenum; microbial remediation; one-factor-at-a-time (OFAT); response surface methodology (RSM)

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1. Introduction

Molybdenum (Mo) is one of the essential elements for living organisms and is needed in small amounts. However, Kulikova et al. [1] pointed out that elevated intake of Mo inhibits the production of several enzymes and causes cell death. Indiscriminate use of Mo in industries has led to irrepressible anthropogenic emission and is a rising concern of pollution in the environment. Affected countries include Japan, Austria, and New Mexico [2]. Antarctica, a virtually uninhabited continent, has also been reported to be polluted by various heavy metals including Mo, and has started to unfavourably affect the ecosystem in Antarctica. This was principally due to the anthropogenic activities in nearby countries including Chile, as it has become one of the largest Cu-Mo producers in the world [3]. Yang et al. [4] exposed that lakes at Taylor Valley, Antarctica, are likely to have

an increasing amount of Mo as the depth increased with 5.05 nmol/kg and 43 nmol/kg in Lake Hoare, whereas it increased between 3.52 nmol/kg and 25.5 nmol/kg in Lake Fryxell.

In the last few decades, chemical precipitation and ion exchange have been used to remove heavy metals from the environment [5]. However, bioremediation is a more effective method for polluted land and water and is more ecologically sustainable [6]. Bioremediation of Mo involves a complex enzymatic reduction process by reducing sodium molybdate (6+) to Mo-blue, a colloid with oxidation states of 5+ [7,8]. In solution, Mo6+ exists as molybdate ion, $[MoO_4]^{2-}$ [9]. Under acidic conditions, $[MoO_4]^{2-}$ can form various polyions such as $Mo_7O_{24}{}^{6-}$, $Mo_8O_{26}{}^{4-}$, and $Mo_{12}O_{37}{}^{2-}$, which can be reduced by reducing agents or combined with many heteroatoms such as phosphate [10]. The combined Mo polyions from molybdophosphate can be reduced into intense blue, colloidal product, heteropolymolybdenum blue. The production of this colloid is crucial as it can be physically removed from solution using dialysis tubing [11]. Komori et al. [12] were the first to report on the bioremoval of chromate using dialysis tubing. The dialysis tubing method is an attractive removal system, as other immobilising systems tend to clog due to the cell mass and reduced heavy metal precipitates. Halmi et al. [13] reported on the use of this method in Mo-blue removal.

Many tropical Mo-reducing bacterial strains, such as *Klebsiella* sp. and *Burkholderia* sp., have been reported in the past few decades. However, only a few isolates from the cold region have been identified in this recent decade [8,14]. *Pseudomonas* sp. strain DRY1 was the first and only Antarctic bacterium isolated from soil reported to have the potential to remediate Mo pollution [8]. Ahmad et al. [8] managed to partially purify the Mo-reducing enzyme from the strain DRY1, but the study was done only in controlled laboratory conditions and had not been carried out in the natural Antarctic environment. Hence, a study on a cold-tolerant bacterial strain able to reduce Mo and produce greater Mo-blue after several optimisation processes will help in overcoming Mo pollution in Antarctica.

Antarctic soil bacterium, *Arthrobacter* sp. strain AQ5-05, was selected for this study. This strain has been previously reported to be able to degrade diesel and phenol [15,16]. It is an aerobic, gram-positive, non-motile, and non-spore-forming bacterium with a rod-coccus growth cycle. The colonies have been described as yellow and translucent and able to grow at temperatures up to 25 °C. The main objectives of this study are to determine the capability of bacterial strain *Arthrobacter* sp. AQ5-05 in reducing Mo and to generate the most effective condition for Mo reduction via OFAT approach. Conditions such as pH, temperature, types of electron donor, and salinity have been reported to affect the efficiency of Mo reduction [8,17]. This study also investigates the relationship between the physicochemical variables for a more efficient Mo reduction via RSM approach.

2. Materials and Methods

2.1. Sample Collection and Maintenance

A bacterium strain *Arthrobacter* sp. strain AQ5-05 was isolated from the Antarctic soil sample collected from King George Island, South Shetland Islands, Antarctica (62°09′7.2″ S, 58°11.4″ W). The nucleotide sequence for this strain has previously been deposited in the NCBI database under the accession number KX946130 [16]. Pure culture of the strain was maintained in 80% glycerol and stored in −80 °C freezer in Eco-Remediation Technology Laboratory, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

2.2. Screening the Bacterial Strain for Mo-Reducing Potential

Molybdenum-supplemented low phosphate media (LPM) (pH 7.0) was prepared by adding (%) glucose (1.0), magnesium sulphate pentahydrate ($MgSO_4.7H_2O$) (0.05), ammonium sulphate ($(NH_4)_2SO_4$) (0.3), sodium chloride (NaCl) (0.5), sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$) (0.242), yeast extract (0.05), and disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) (0.05) [17]. The media and glucose were separately autoclaved at 121 °C for 15 min. An aliquot of bacterial culture *Arthrobacter* sp. strain AQ5-05 (10% *v*/*v*) was incubated in LPM at 10 °C on 150 rpm orbital shaker to identify its reduction

potential. The blue intensity of Mo-blue produced was observed daily at optical density (OD) of 865 nm wavelength using a UV-VIS spectrophotometer [18], while the bacterial growth was observed by counting the number of colonies spread on nutrient agar (NA) using a spread plate technique and expressed in terms of Log CFU/mL.

2.3. Optimisation of Mo Reduction

2.3.1. One-Factor-at-a-Time (OFAT)

Preliminary optimisation using one-factor-at-a-time (OFAT) was used to observe the optimum condition of Mo reduction of strain AQ5-05 based on eight parameters, which are types of carbon source (fructose, sucrose, sorbitol, lactose, glucose, maltose), carbon source concentrations (0.0 to 5.0 g/L), types of nitrogen source (glycine, urea, ammonium acetate, ammonium chloride, acrylamide, ammonium sulphate), nitrogen source concentrations (0.0 to 0.6 g/L), salinity (0.0 to 0.6 g/L), molybdate concentration (0 to 30 mM), pH (5 to 8.5), and temperature (10 to 30 °C). The control group for two variables, types of carbon and nitrogen source, were indicated by the absence of carbon and nitrogen in the media, respectively. Bacteria culture of 1 mL was inoculated into 10 mL of LPM (10% v/v). Each parameter was conducted in triplicates. The sample was left for seven days at 150 rpm on an orbital shaker at 10 °C. The evaluation on the production of Mo-blue and bacterial growth of each parameter followed the procedures described in Section 2.2.

2.3.2. Response Surface Methodology (RSM)

Further optimisation was carried out using a statistical RSM. RSM is a tool that explores the interaction between each parameter affecting the Mo-reducing ability of *Arthrobacter* sp. strain AQ5-05 by running sequential sets of experiment [17]. In this part of the study, Box-Wilson central composite design (CCD) was implemented to screen the variables using Design-Expert Version 6.0.8 (Stat-Ease Inc., Minneapolis, MN, USA). CCD was employed to construct the response surface from the selected parameters. The effect of each of these parameters on Mo reduction was analysed at two axial points, two factorial points, and a single central point (+2/−2, +1/−1, and 0, respectively), as shown in Table 1 [19].

Table 1. Experimental range and level of independent variables tested in CCD.

Variables	Symbol	Unit	Experimental Level				
			−2	−1	0	+1	+2
Sucrose concentration	A	g/L	0.09	1	1.5	2	2.91
$(NH_4)_2SO_4$ concentration	B	g/L	0.11	0.2	0.25	0.3	0.39
Salinity	C	g/L	0.41	0.5	0.55	0.6	0.69
Molybdate concentration	D	g/L	5.43	10.0	12.5	15.0	19.57
pH	E	-	6.54	7.0	7.25	7.5	7.96
Temperature	F	°C	5.4	10.0	12.5	15.0	19.6

3. Results and Discussion

3.1. Screening of the Mo-Reducing Potential of Arthrobacter sp. Strain AQ5-05

As shown in Figure 1, the highest Mo reduction of *Arthrobacter* sp. strain AQ5-05 is on the eighth day of incubation at 1.845 and bacterial growth at 11.043 Log CFU/mL. A study by Ahmad et al. [8] using *Pseudomonas* sp. strain DRY1 isolated from Antarctic soil have shown to be able to reduce molybdenum optimally after 72 h. To date, *Arthrobacter* sp. strain AQ5-05 is the first *Arthrobacter* genus from Antarctic that has shown the ability to reduce Mo.

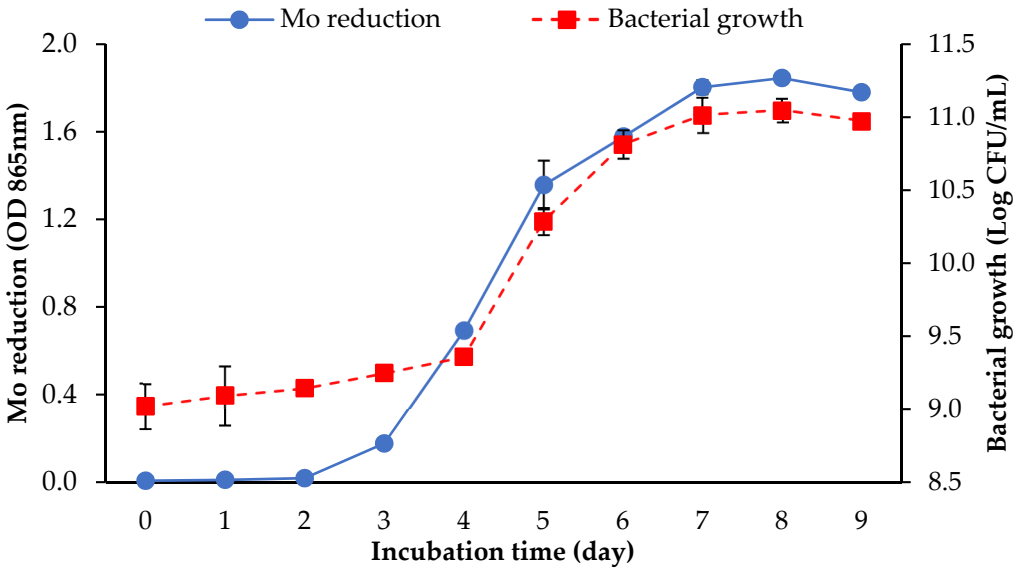

Figure 1. Molybdate reduction and growth of *Arthrobacter* sp. strain AQ5-A5 isolate in LPM.

3.2. Optimisation of Mo Reduction Using One-Factor-at-a-Time (OFAT)

3.2.1. Carbon Source Concentration

Figure 2a shows that the two carbon sources, namely glucose and sucrose, supported the molybdate reduction. However, sucrose slightly was favoured by strain AQ5-05 for Mo reduction ($p < 0.001$), as analysed using Tukey's multiple comparison test. Subsequently, a series of sucrose concentrations ranging from 0 to 5 (g/L) were tested to find the optimum concentration. As shown in Figure 2b, the Mo-blue production and growth were optimum at concentration 1 g/L after 168 h with no significant differences with 2 g/L (Tukey's multiple comparison tests, $p > 0.05$), as analysed using ANOVA. 1 g/L sucrose was selected for subsequent experiment due to the cost-effectiveness factor for bioremediation.

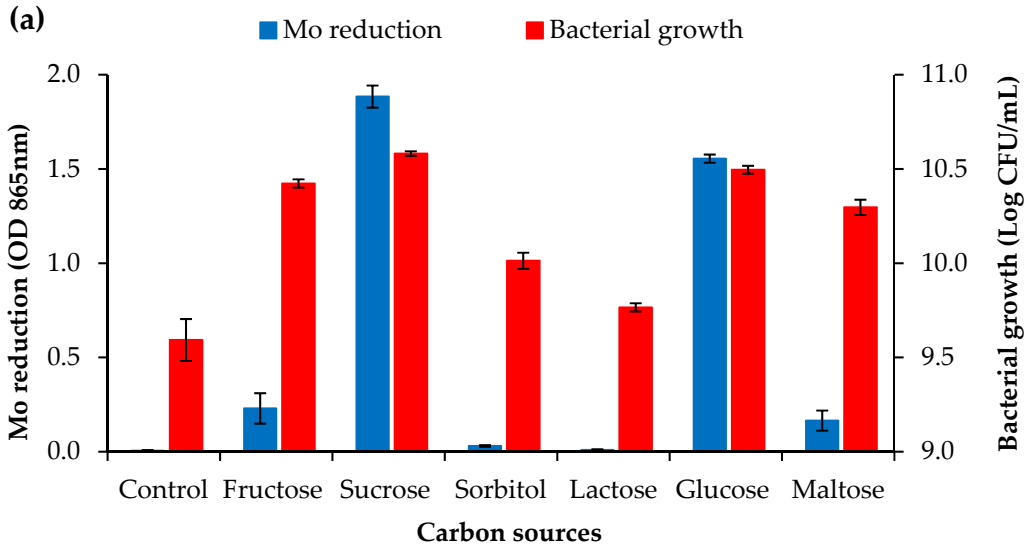

Figure 2. *Cont.*

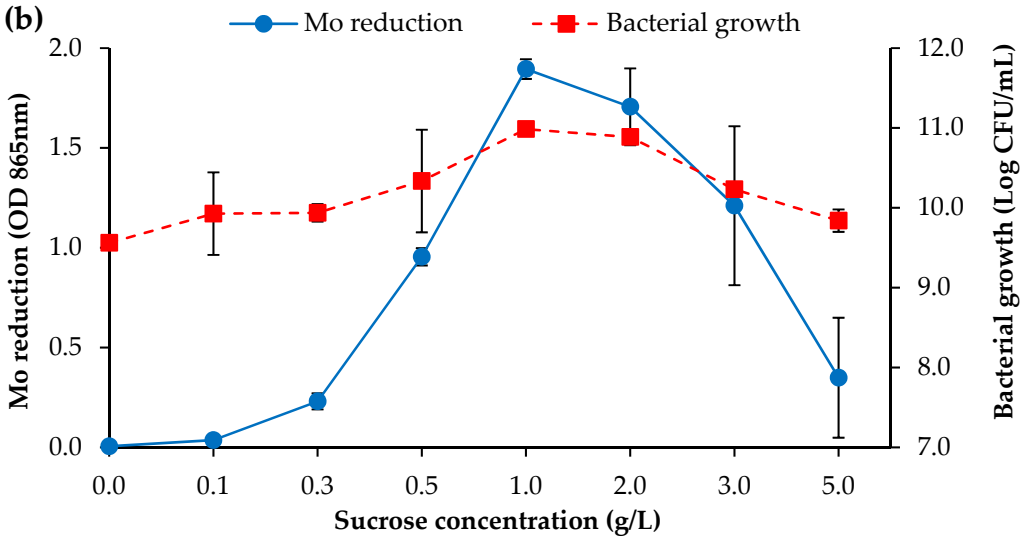

Figure 2. (**a**) The effects of various electron donors at the final concentration (*w*/*v*) of 1% on bacterial growth and Mo reduction; (**b**) The effects of different sucrose concentration on Mo reduction and growth of strain AQ5-05 in LPM after 168 h at 10 °C. The error bars represent the mean ± standard deviation for three replicates.

In the Mo reduction metabolic pathway, simple carbohydrates are preferred as electron donors as they can produce reducing equivalents for NADH and NADPH and are the substrates for Mo-reducing enzymes [20,21]. A few mesophilic bacterial strains such as *Bacillus* sp. strain Neni-10, *Klebsiella oxytoca* strain Saw-5, *Enterobacter* sp. strain Saw-1, *Burkholderia vietnamiensis* strain AQ5-12, and *Burkholderia* sp. strain AQ5-13 have been reported to favour both glucose and sucrose as the best electron donors for Mo reduction, though glucose was favoured more than sucrose except for *Burkholderia* sp. strain AQ5-13 [2,6,22,23]. Low ODs seen in other carbon sources, especially lactose, maltose, and sorbitol, ascertain the incapability of these sugars to produce reducing equivalents for NADH and NADPH.

The preferability of simple sugars such as glucose and sucrose in Mo reduction might also be because Mo reduction is growth-associated; therefore, the use of easily assimilable sugar is more favourable [6]. In comparison with a study by Ahmad et al. [7] using *Pseudomonas* sp. strain DRY1 isolated from Antarctic soil, glucose was the best electron donor, while Darham et al. [17] reported that Mo-reducing Antarctic marine bacterium *Marinomonas* sp. strain AQ5-A9 favour sucrose, thereby reflecting a simple growth-associated process of the species.

3.2.2. Nitrogen Sources and Concentration

Figure 3a shows that ammonium sulphate ($(NH_4)_2SO_4$) significantly produced the highest OD and bacterial growth (Tukey's multiple comparison test, all $p < 0.001$). Other nitrogen sources such as urea and glycine did not support Mo-blue production, but acrylamide showed low Mo-blue production. Subsequently, a series of different concentrations of $(NH_4)_2SO_4$ ranging from 0.1 to 0.6 g/L were assessed to find the optimum concentration. Figure 3b indicates that the optimum Mo reduction and the highest bacterial growth were observed at 0.3 g/L.

Ahmad et al. [8] stated that $(NH_4)_2SO_4$ is an easily assimilable nitrogen source along with two ammonium ions to be used. Furthermore, its availability to be commonly utilised by bacteria and affordable for practical bioremediation have made $(NH_4)_2SO_4$ a good nitrogen source [7]. Previous work on *Marinomonas* sp. strain AQ5-A9, *Serratia* sp. strain DRY5, *Burkholderia vietnamiensis* strain AQ5-12, *Burkholderia* sp. strain AQ5-13, and *Bacillus* sp. strain A.rzi demonstrated that these bacteria work best in $(NH_4)_2SO_4$ [17,23–25].

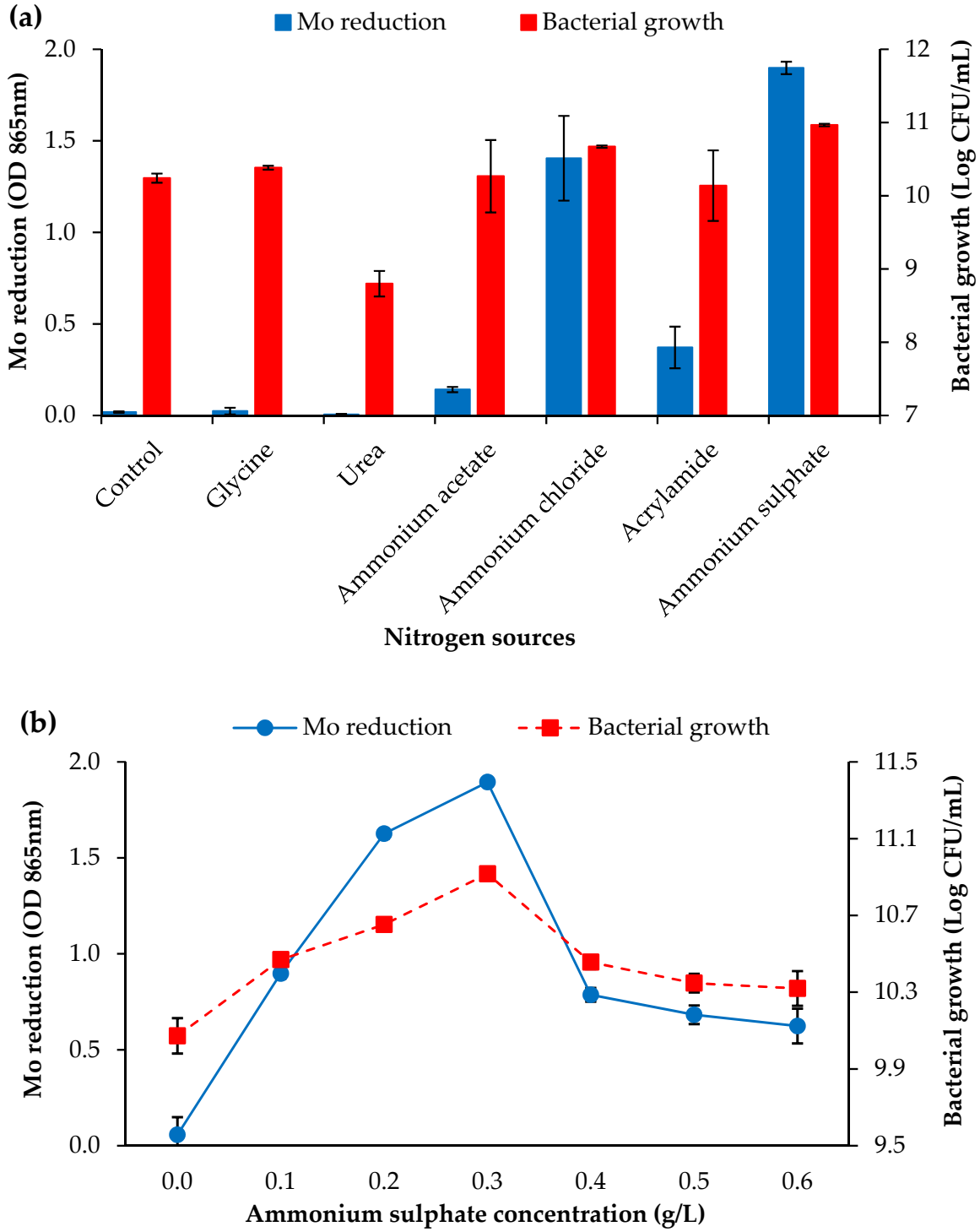

Figure 3. (**a**) The effects of different nitrogen sources at an initial concentration of 0.3 g/L; (**b**) the effect of different concentrations of $(NH_4)_2SO_4$ on Mo reduction and growth of strain AQ5-05 in LPM. The error bars represent the mean ± standard deviation for three replicates.

3.2.3. Salinity

Figure 4 depicts that 0.5 g/L of NaCl was the optimum salinity for strain AQ5-05 to grow and reduce Mo, as it shows the highest OD and growth compared to other concentrations (Tukey's test, $p < 0.001$).

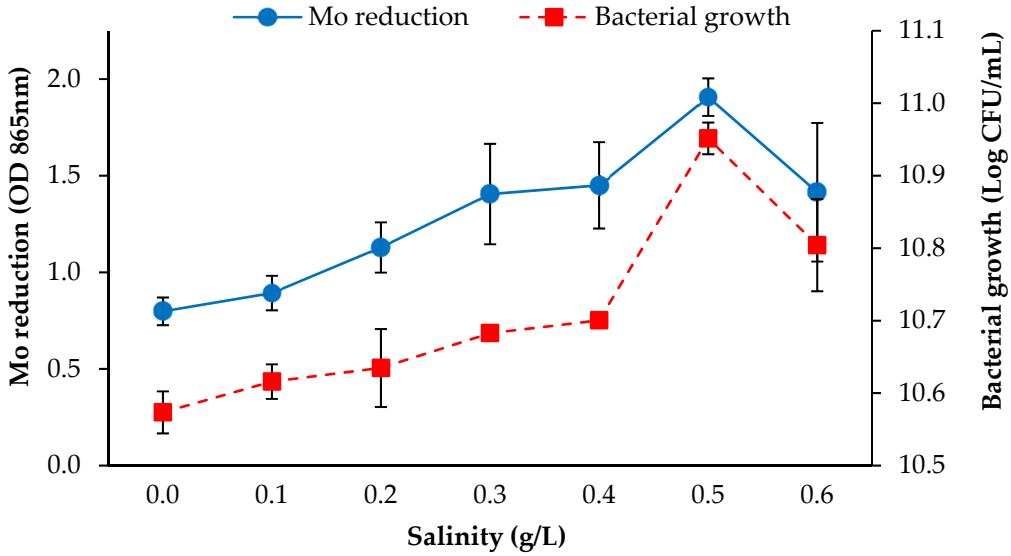

Figure 4. The effects of salinity on Mo reduction and growth activity of strain AQ5-05. The error bars represent the mean ± standard deviation for three replicates.

According to Lee et al. [16], salt content and composition of Antarctic soil are known to vary most obviously with proximity to the coast or to dense colonies of marine vertebrates. Understanding the limiting nutrients by incorporating the appropriate amount of NaCl used is a favourable strategy in increasing the formation of Mo-blue, as high salinity beyond the tolerant level can disrupt the osmotic balance in a microorganism [16]. Furthermore, high salinity tolerance is suitable for marine bioremediation, while low salinity tolerance suggests bioremediation in soil or freshwater. This can be confirmed when a marine bacterium, *Marinomonas* sp. strain AQ5-A9, and a soil bacterium, *Pseudomonas* sp. strain DRY1, have contrasting optimum salinity of 47 g/L and 5 g/L of NaCl, respectively [8,17].

3.2.4. Molybdate Concentration

Figure 5 illustrates that the molybdate concentration showed the highest, ranging from 5 to 15 mM. The highest OD and bacterial growth can be seen at 10 mM. The reduction of Mo started to decrease at a much higher Mo concentration, while the bacterial growth started to steadily decline as the concentration is more than 20 mM. Molybdate concentration is the key player in determining the success of bioremediation, as it is one of the anions with the ability to inhibit Mo-blue production in bacteria [6]. To date, reports on Mo reduction demonstrated the highest concentration achieved by an Antarctic bacterium, *Pseudomonas* sp. strain DRY1, which is at 50 mM [8], while Antarctic marine bacterium, *Marinomonas* sp. strain AQ5-A9, has a lower tolerance towards Mo and was able to reduce Mo optimally at 15 mM. However, reduction still occurs as concentration reaches up to 40 mM [17]. Mo contamination in the environment has been accounted to reach 20.8 mM molybdate (2000 ppm) [26,27]. Hence, resistance higher than 20 mM is an advantage for an organism, as this concentration is lethal to the ruminant [28].

3.2.5. pH

Three buffers were applied in this system: acetate buffer (for pH 5, 5.5, and 6), phosphate buffer (for pH 6.0, 6.5, 7.0, and 7.5), and Tris-HCl buffer (for pH 7.5, 8.0, and 8.5). Figure 6 shows that phosphate buffer pH 7 was the most desirable pH for the formation of Mo-blue and bacterial growth. Finding the right pH is crucial, as the slightest change in pH has impact on microbial metabolic activity. When pH is deviated from neutral conditions, the degradation rate slows down.

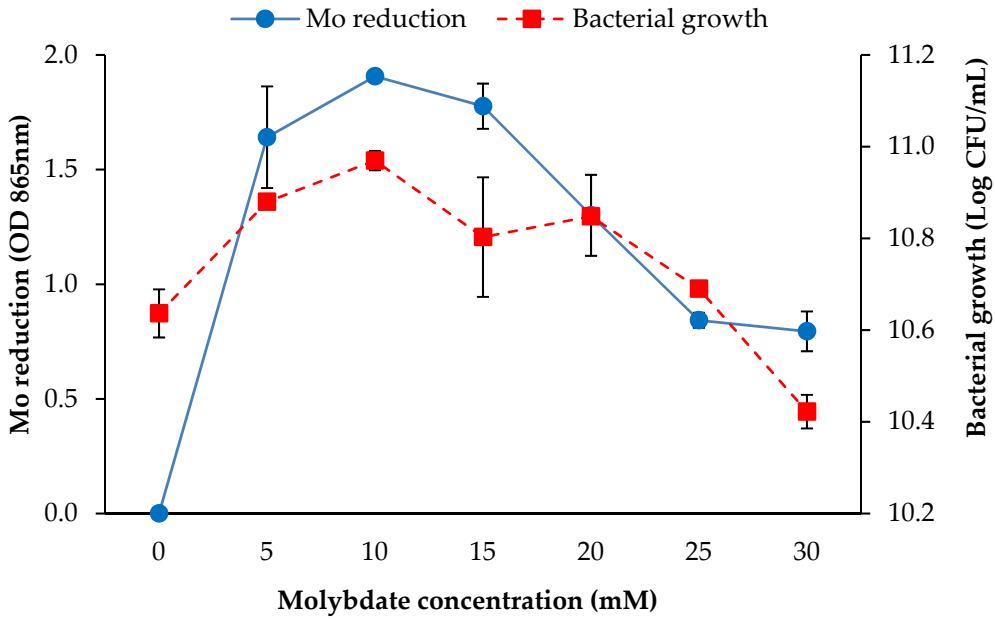

Figure 5. The effects of molybdate concentration on Mo reduction and growth activity of strain AQ5-05. The error bars represent the mean ± standard deviation for three replicates.

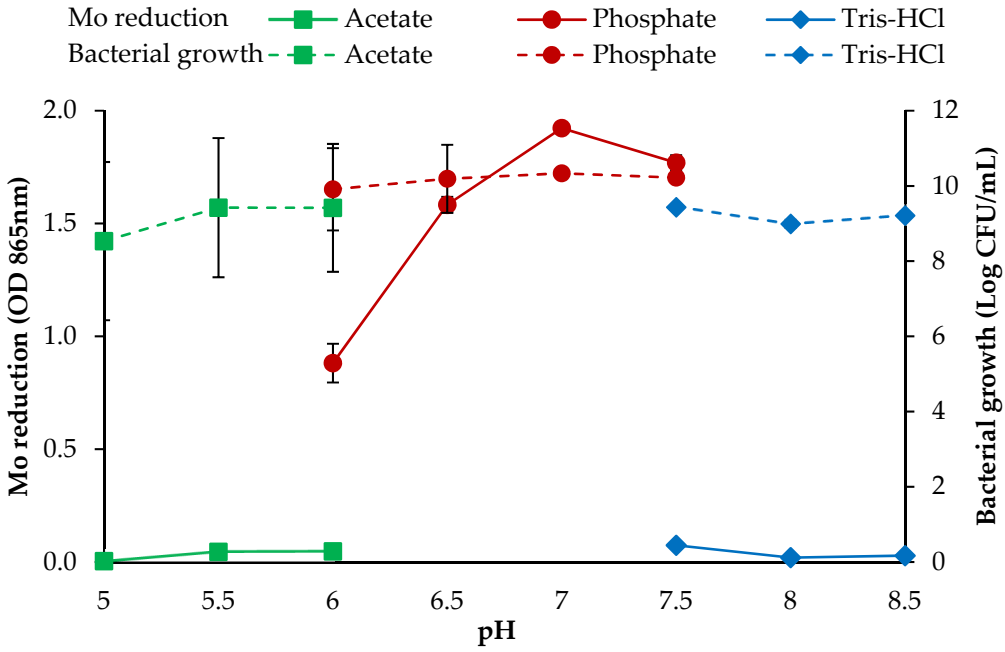

Figure 6. The effects of pH on Mo reduction and growth of strain AQ5-05. The error bars represent the mean ± standard deviation for three replicates.

pH affects microbial metabolisms, mainly by controlling the kinetics of redox reactions [29]. The optimal pH varies across the individual organisms and their ability to intracellularly control acid and base levels [17]. Lee et al. [16] has reported that *Arthrobacter* sp. strain AQ5-05 is a neutrophilic bacterium with growing characteristics between pH 5.5 and 8 with an optimum pH of 7.5 in phenol degradation. Previous studies regarding Mo reduction have shown that the optimum pH is slightly acidic to neutral as phosphomolybdate is highly unstable in alkaline conditions [8,21,30,31]. In phosphate buffer, molybdate can be converted effectively to phosphomolybdates, thus increasing the production of Mo-blue [22]. *Bacillus* sp. strain A.rzi and *Klebsiella oxytoca* strain DRY14 have an optimal pH of 7 [11,25], while some strains prefer slightly lower pH that supports optimal Mo

reduction, such as between 6.5 and 6.8 for *Enterobacter* sp. strain SAW-2 and 5.8 and 6.8 for *Bacillus* sp. strain Khayat [2,32].

3.2.6. Temperature

Temperature is the most important physical factor in the Mo bioremediation process, as it is a process mediated by a biological enzyme which has an optimum temperature [8,32]. It is important to search for an optimum temperature appropriate for indigenous microbes [17]. Figure 7 shows that 10 °C was the highest temperature for Mo reduction and bacterial growth. The reduction activity plummets as the temperature increases and the Mo-blue production halts as the temperature reaches 20 °C, while bacterial growth declines steadily with the increase in temperature. Previous reports stated that most Antarctic bacteria are psychrotolerant rather than strictly psychrophilic [8,16,33,34]. Therefore, most native microorganisms can withstand high temperature fluctuations considering the weather and climate of the continent.

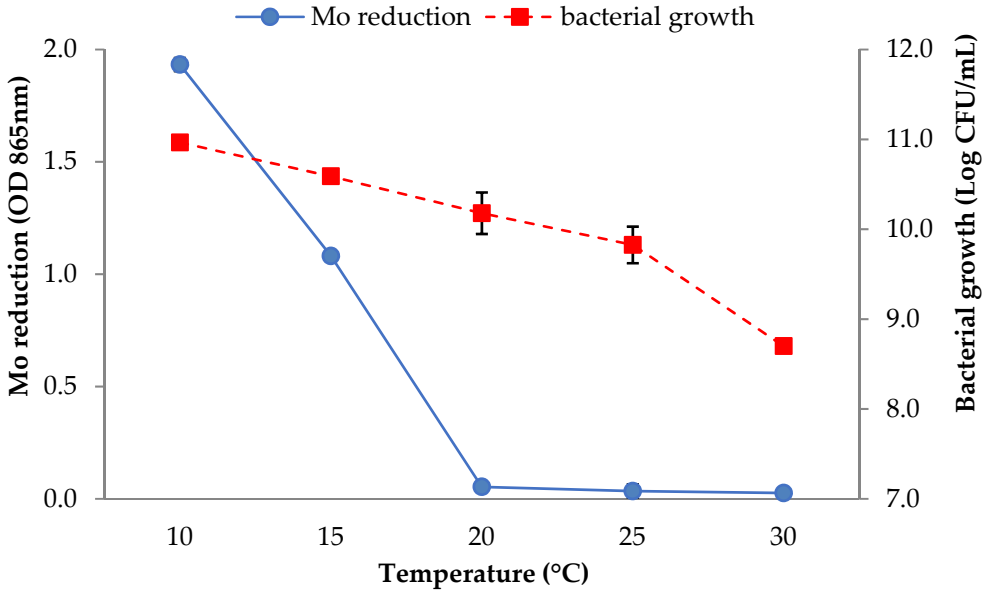

Figure 7. The effects of temperature on Mo reduction and growth of strain AQ5-05. The error bars represent the mean ± standard deviation for three replicates.

A previous study on strain AQ5-05 on phenol degradation by Lee et al. [16] showed an optimum temperature between 10 °C and 15 °C. A Mo-reducing study using Antarctic bacterium *Marinomonas* sp. AQ5-A9 showed an optimum temperature between 15 to 20 °C, confirming its psychrotolerant attribute. On the contrary, bacterial strains isolated from tropical countries have a much higher temperature for optimal Mo reduction, such as *Bacillus* sp. strain Khayat having an optimum temperature between 25 °C to 34 °C, 28 °C to 30 °C for *Bacillus* sp. strain A.rzi, 30 °C to 40 °C for *Burkholderia vietnamiensis* strain AQ5-12, and 25 °C for *Klebsiella oxytoca* strain DRY14 [11,23,25,32].

3.3. Optimisation of Mo Reduction Using Response Surface Methodology (RSM)

3.3.1. Central Composite Design (CCD)

CCD is an experimental design in RSM employed to optimise the parameters from OFAT. Eighty-six experimental runs of six parameters were incorporated into the CCD analysis. Table 2 shows the CCD validated by ANOVA. The *F* value of the model was 39.04 with 'Prob > *F*' values of less than 0.05, indicating that the model was significant in Mo reduction process. The significant lack of fit denoted that the model fits the data [35]. In this case, the linear terms A, C, D, E, F, quadratic terms A^2 to F^2, and interactive terms AB, AD, AF, BC, BF, CD, CF, DE, DF, EF were significant. The ANOVA illustrated the R^2 for

this model by 0.948, whereas the 'Pred-R^2' value of 0.8701 was in reasonable agreement with the 'Adj-R^2' of 0.9802 and 0.9576 for Mo reduction.

Table 2. Analysis of variance (ANOVA) for Mo reduction in CCD.

Source	Sum of Squares	df	Mean Square	*F* Value	Prob > *F*
Model	10.25654	27	0.379872	39.04075	<0.0001 *
A	1.533952	1	1.533952	157.6496	<0.0001 *
B	0.000928	1	0.000928	0.095385	0.7585
C	0.125828	1	0.125828	12.93182	0.0007 *
D	0.506057	1	0.506057	52.00924	<0.0001 *
E	1.161845	1	1.161845	119.4068	<0.0001 *
F	2.791166	1	2.791166	286.8578	<0.0001 *
A^2	1.58817	1	1.58817	163.2217	<0.0001 *
B^2	0.684594	1	0.684594	70.35808	<0.0001 *
C^2	0.767384	1	0.767384	78.86666	<0.0001 *
D^2	1.128975	1	1.128975	116.0287	<0.0001 *
E^2	0.2677	1	0.2677	27.51244	<0.0001 *
F^2	0.769687	1	0.769687	79.10336	<0.0001 *
AB	0.054231	1	0.054231	5.573483	0.0216 *
AC	0.033535	1	0.033535	3.446484	0.0685
AD	0.065344	1	0.065344	6.715643	0.0121 *
AE	0.012183	1	0.012183	1.252052	0.2678
AF	0.08316	1	0.08316	8.546655	0.0049 *
BC	0.063315	1	0.063315	6.507116	0.0134 *
BD	0.011854	1	0.011854	1.218252	0.2743
BE	0.003525	1	0.003525	0.362317	0.5496
BF	0.049562	1	0.049562	5.093647	0.0278 *
CD	0.055284	1	0.055284	5.681704	0.0204 *
CE	0.005532	1	0.005532	0.568506	0.4539
CF	0.058746	1	0.058746	6.037493	0.0170 *
DE	0.072025	1	0.072025	7.402273	0.0086 *
DF	0.236561	1	0.236561	24.31215	<0.0001 *
EF	0.069498	1	0.069498	7.142564	0.0098 *
Residual	0.564348	58	0.00973		
Lack of Fit	0.469043	49	0.009572	0.903946	0.6238
Pure Error	0.095305	9	0.010589		
Cor Total	10.82089	85			
Std. Dev.	0.098641		*R*-Squared		0.948
Mean	1.250234		Adj *R*-Squared		0.9236
C.V.	7.889842		Pred *R*-Squared		0.87013
PRESS	1.405346		Adeq Precision		27.37344

A: Sucrose concentration (g/L); B: $(NH_4)_2SO_4$ concentration (g/L); Salinity (g/L); D: Molybdate concentration (g/L); E: pH; F: Temperature (°C). * Significant.

Therefore, the model was suitable for Mo-blue prediction and final equation in terms of coded factors, as illustrated in Equation (1) in which *Y* is Mo reduction.

$$Y = +1.73 - 0.14A - 3.406E^{-003}B + 0.040C - 0.080D - 0.12E^{-0.19}F - 0.12A^2 - 0.079B^2 - 0.083C^2 - 0.10D^2 - 0.049E^2 - 0.083F^2 - 0.029AB - 0.023AC + 0.032AD - 0.014AE - 0.036AF + 0.031BC + 0.014BD + 7.422E^{-003}BE - 0.028BF + 0.029CD - 9.297E^{-003}CE - 0.030CF + 0.034DE + 0.061DF - 0.033EF \quad (1)$$

CCD response was used to create 3D surfaces to reveal the significant interactions between two factors while maintaining the others at constant level [36]. Figure 8 shows the 3D contour plot of the significant interaction terms AB, AD, AF, BC, BF, CD, CF, DE, DF, EF.

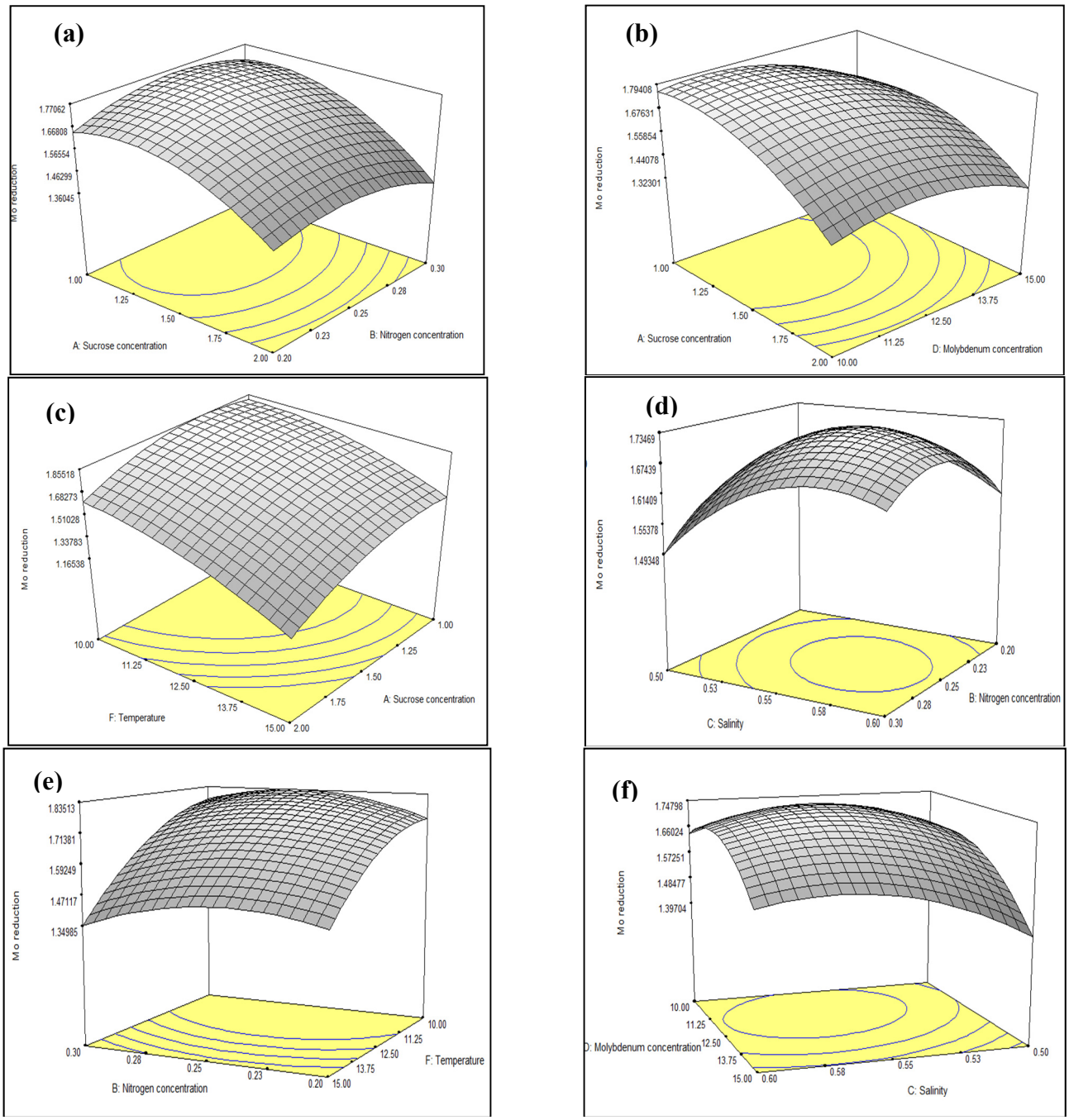

Figure 8. *Cont*.

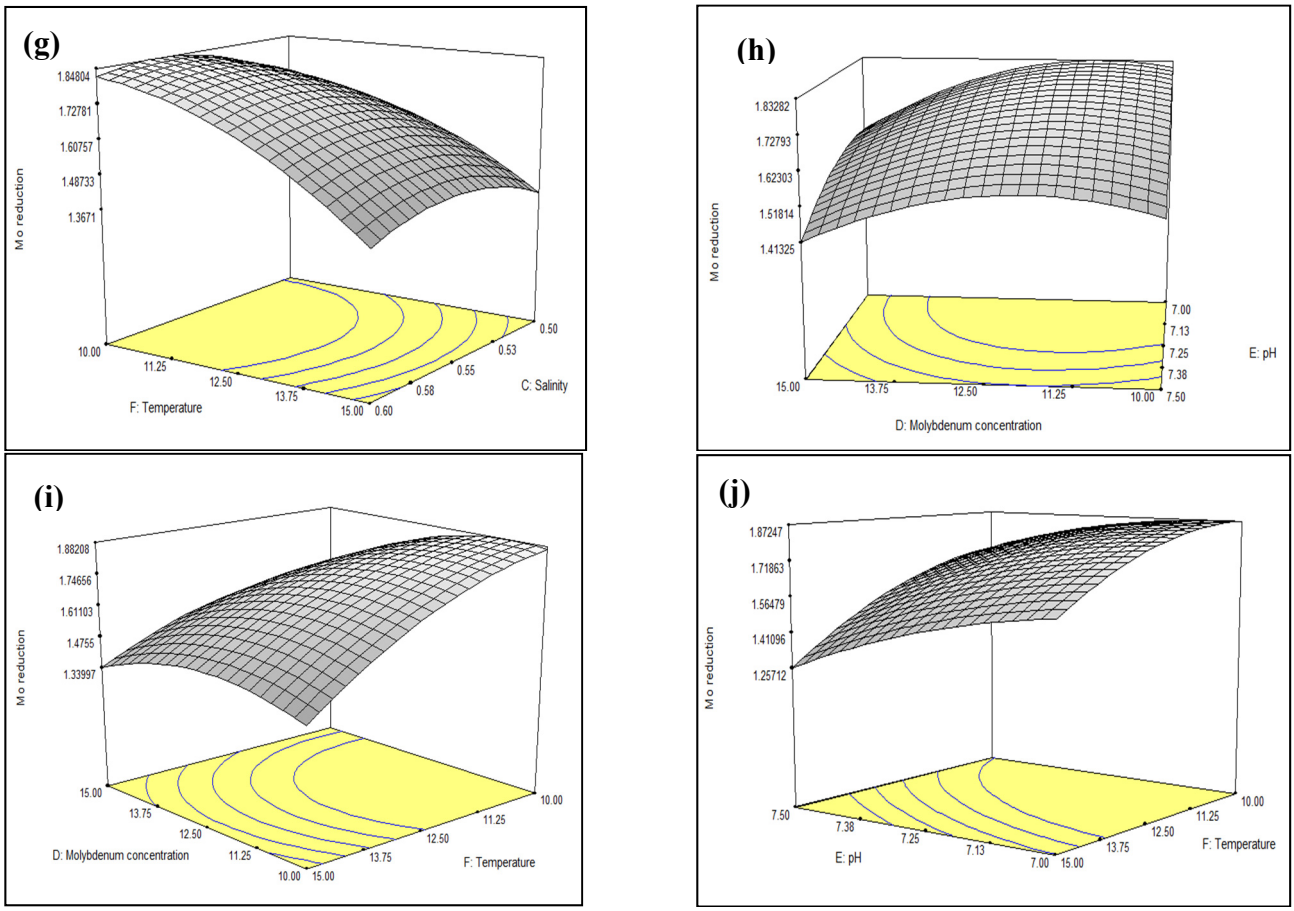

Figure 8. Three-dimensional contour plot showing the effect of interactions between a pair of parameters on Mo reduction by strain AQ5-05. (**a**) AB; (**b**) AD; (**c**) AF; (**d**) BC; (**e**) BF; (**f**) CD; (**g**) CF; (**h**) DE; (**i**) DF; (**j**) EF.

3.3.2. Model Prediction and Validation

From the acquired data, Table 3 shows the optimal conditions predicted by RSM, which resulted in Mo reduction of OD 2.1 as measured at 865 nm. Validation was done by following the optimised value generated by the software, which resulted in an absorbance value of 2.19. Predicted and experimental values were analysed using one-way ANOVA with post hoc analysis by Tukey's test to assess the significance of the model. The *p*-value obtained was more than 0.05, denoting that predicted value and experimental value are not significantly different, hence validating the model.

Table 3. Predicted and experimental value for Mo reduction using *Arthrobacter* sp. strain AQ5-05.

Symbol	Variable	Unit	Generated Value	Mo Reduction ($OD_{865\ nm}$)	
				Predicted	Experimental
A	Sucrose concentration	g/L	1.0	2.1	2.19
B	Nitrogen concentration	g/L	0.25		
C	Salinity	g/L	0.55		
D	Molybdate concentration	mM	12.5		
E	pH	-	7.0		
F	Temperature	°C	10.0		

Table 4 shows the comparison of Mo reduction in OFAT and RSM. The Mo reduction was higher in RSM compared to OFAT. RSM helps in understanding the effects of independent factors and the interactions of different parameters, and thus assisted in optimising

the parameters and predicting their response [19]. Yakasai et al. [37] reported that after optimisation using RSM, the response of Mo reduction obtained was more accurate compared to OFAT. Studies by Lee et al. [36] and Yusuf et al. [38] proved that RSM has the advantage over OFAT as it incorporates the interaction between variables and has higher accuracy.

Table 4. Optimised condition obtained using OFAT and RSM for Mo reduction using *Arthrobacter* sp. strain AQ5-05.

Symbol	Variable	Unit	Optimised Value	
			OFAT	RSM
A	Sucrose concentration	g/L	1.0	1.0
B	Nitrogen concentration	g/L	0.20	0.25
C	Salinity	g/L	0.50	0.55
D	Molybdate concentration	mM	10.0	12.5
E	pH	-	7.0	7.0
F	Temperature	°C	10.0	10.0
	Mo reduction (OD 865 nm)		1.93	2.19

4. Conclusions

In this study, *Arthrobacter* sp. strain AQ5-05 demonstrated Mo-reducing capability. Molybdate reduction to Mo-blue by *Arthrobacter* sp. strain AQ5-05 has been successfully optimised using OFAT and RSM approaches. Optimisation using OFAT successfully determined the optimum value and range of each parameter. The CCD incorporates the values from OFAT for further optimisation, as well as observing the interactions between variables under examination. The analysis of linear and quadratic terms, as well as the interactions between significant factors, were identified as having a good agreement with the high R^2 value, proving the feasibility of the model. Knowledge of the various optimised parameters for this bacterium would contribute to an effective translation of the laboratory outcomes to the in-situ application. This study provides information on *Arthrobacter* sp. strain AQ5-05's ability and potential in remediating Mo pollution in Antarctic soil. For future works, an efficient in-situ removal system of Mo-blue precipitates, such as dialysis tubing that would benefit Mo-polluted Antarctic soils and water bodies, could be further investigated.

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References

1. Kulikova, O.I.; Fedorova, T.N.; Lopachev, A.V.; Orlova, V.S.; Grachev, V.A. Effects of antioxidants on the viability of the human neuroblastoma SH-SY5Y cell culture under the conditions of heavy-metal toxicity. *Biol. Med.* **2016**, *8*, 4–8. [CrossRef]
2. Sabullah, M.K.; Rahman, M.F.; Ahmad, S.A.; Sulaiman, M.R.; Shukor, M.S.; Shamaan, N.A.; Shukor, M.Y. Assessing resistance and bioremediation ability of *Enterobacter* sp. strain Saw-1 on molybdenum in various heavy metals and pesticides. *J. Math. Fundam. Sci.* **2017**, *49*, 193–210. [CrossRef]
3. Barra, F.; Alcota, H.; Rivera, S.; Valencia, V.; Munizaga, F.; Maksaev, V. Timing and formation of porphyry Cu-Mo mineralization in the Chuquicamata district, northern Chile: New constraints from the Toki cluster. *Miner. Deposita.* **2013**, *48*, 629–651. [CrossRef]
4. Yang, N.; Welch, K.A.; Mohajerin, T.J.; Telfeyan, K.; Chevis, D.A.; Grimm, D.A.; Lyons, W.B.; White, C.D.; Johannesson, K.H. Comparison of arsenic and molybdenum geochemistry in meromictic lakes of the McMurdo Dry Valleys, Antarctica: Implications for oxyanion-forming trace element behavior in permanently stratified lakes. *Chem. Geol.* **2015**, *404*, 110–125. [CrossRef]
5. Barakat, M.A. New trends in removing heavy metals from industrial wastewater. *Arab. J. Chem.* **2011**, *4*, 361–377. [CrossRef]
6. Mansur, R.; Gusmanizar, N.; Roslan, M.A.; Ahmad, S.A.; Shukor, M.Y. Isolation and characterisation of a molybdenum-reducing and metanil yellow dye-decolourising *Bacillus* sp. strain neni-10 in soils from West Sumatera, Indonesia. *Trop. Life Sci. Res.* **2017**, *28*, 69–90. [CrossRef]
7. Sabullah, M.K.; Rahman, M.F.; Ahmad, S.A.; Sulaiman, M.R.; Shukor, M.S.; Shamaan, N.A.; Shukor, M.Y. Isolation and characterization of a molybdenum-reducing and phenolic- and catechol-degrading *Enterobacter* sp. strain saw-2. *Biotropia* **2017**, *24*, 47–58. [CrossRef]
8. Ahmad, S.A.; Shukor, M.Y.; Shamaan, N.A.; Mac Cormack, W.P.; Syed, M.A. Molybdate reduction to molybdenum blue by an Antarctic bacterium. *BioMed Res. Int.* **2013**, *2013*. [CrossRef]
9. Lee, J.D. *Concise Inorganic Chemistry*; Van Reinhold Co.: New York, NY, USA, 1977; p. 325.
10. Braithwaite, E.R. Molybdenum. In *Specialty Inorganic Chemicals*; Thompson, R., Ed.; The Royal Society of Chemistry, Burlington House: London, UK, 1981; pp. 350–351.
11. Halmi, M.I.E.; Zuhainis, S.W.; Yusof, M.T.; Shaharuddin, N.A.; Helmi, W.; Shukor, Y.; Syed, M.A.; Ahmad, S.A. Hexavalent molybdenum reduction to Mo-blue by a sodium-dodecyl-sulfate-degrading *Klebsiella oxytoca* strain DRY14. *BioMed Res. Int.* **2013**, *2013*. [CrossRef]
12. Komori, K.; Rivas, A.; Toda, K.; Ohtake, H. A method for removal of toxic chromium using dialysis-sac cultures of a chromate-reducing strain of *Enterobacter cloacae*. *Appl. Microbiol. Biotechnol.* **1990**, *33*, 117–119. [CrossRef]
13. Halmi, M.I.E.; Wasoh, H.; Sukor, S.; Ahmad, S.A.; Yusof, M.T.; Shukor, M.Y. Bioremoval of molybdenum from aqueous solution. *Int. J. Agric. Biol.* **2014**, *16*, 848–850.
14. Darham, S.; Gomez-Fuentes, C.; Zulkharnain, A.; Sabri, S.; Calisto-Ulloa, N.; Ramírez-Moreno, N.; Ahmad, S.A. Isolation and identification of molybdenum-reducing cold-adapted marine bacteria isolated from Bernardo O'Higgins Riquelme base station, Antarctica. *Malays. J. Biochem. Mol. Biol.* **2019**, *22*, 8–15.
15. Abdulrasheed, M.; Zulkharnain, A.; Zakaria, N.N.; Roslee, A.F.A.; Khalil, K.A.; Napis, S.; Convey, P.; Gomez-Fuentes, C.; Ahmad, S.A. Response surface methodology optimization and kinetics of diesel degradation by a cold-adapted Antarctic bacterium, *Arthrobacter* sp. strain AQ5-05. *Sustainability* **2020**, *12*, 6966. [CrossRef]
16. Lee, G.L.Y.; Ahmad, S.A.; Yasid, N.A.; Zulkharnain, A.; Convey, P.; Johari, W.L.W.; Alias, S.A.; Gonzalez-Rocha, G.; Shukor, M.Y. Biodegradation of phenol by cold-adapted bacteria from Antarctic soils. *Polar. Biol.* **2018**, *41*, 553–562. [CrossRef]
17. Darham, S.; Zahri, K.N.M.; Zulkharnain, A.; Sabri, S.; Gomez-Fuentes, C.; Convey, P.; Khalil, K.A.; Ahmad, S.A. Statistical Optimisation and Kinetic Studies of Molybdenum Reduction Using a Psychrotolerant Marine Bacteria Isolated from Antarctica. *J. Mar. Sci. Eng.* **2021**, *9*, 648. [CrossRef]
18. Campbell, A.M.; Campillo-Campbell, A.D.; Villaret, D.B. Molybdate reduction by *Escherichia coli* K-12 and its chl mutants. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 227–231. [CrossRef]
19. Khuri, A.I.; Mukhopadhyay, S. Response surface methodology. *Wiley Interdiscip. Rev. Comput. Stat.* **2010**, *2*, 128–149. [CrossRef]
20. Ghani, B.; Takai, M.; Hisham, N.Z.; Kishimoto, N.; Ismail, A.K.M.; Tano, T.; Sugio, T. Isolation and characterization of a Mo6+ reducing bacterium. *Appl. Environ. Microbiol.* **1993**, *59*, 1176–1180. [CrossRef] [PubMed]
21. Adnan, A.S.M.; Zeid, I.M.A.; Ahmad, S.A.; Halmi, M.I.E.; Abdullah, S.R.S.; Masdor, N.A.; Shukor, M.S.; Shukor, M.Y. A molybdenum-reducing Bacillus sp. strain Zeid 14 in soils from Sudan that could grow on amides and acetonitrile. *Malays. J. Soil Sci.* **2016**, *20*, 111–134.
22. Sabullah, M.K.; Rahman, M.F.; Ahmad, S.A.; Sulaiman, M.R.; Shukor, M.S.; Shamaan, N.A.; Shukor, M.Y. Isolation and characterization of a molybdenum-reducing and glyphosate-degrading *Klebsiella oxytoca* strain saw-5 in soils from Sarawak. *J. Agric. Sci.* **2016**, *38*, 1–13. [CrossRef]
23. Manogaran, M.; Ahmad, S.A.; Yasid, N.A.; Yakasai, H.M.; Shukor, M.Y. Characterisation of the simultaneous molybdenum reduction and glyphosate degradation by *Burkholderia vietnamiensis* AQ5-12 and *Burkholderia* sp. AQ5-13. *3 Biotech* **2018**, *8*, 1–8. [CrossRef]
24. Mansur, R.; Gusmanizar, N.; Dahalan, F.A.; Masdor, N.A.; Ahmad, S.A.; Shukor, M.S.; Roslan, M.A.H.; Shukor, M.Y. Isolation and characterization of a molybdenum-reducing and amide-degrading *Burkholderia* sp. strain neni-11 in soils from West Sumatera, Indonesia. *IIOAB J.* **2016**, *7*, 28–40.
25. Othman, A.R.; Bakar, N.A.; Halmi, M.I.E.; Johari, W.L.W.; Ahmad, S.A.; Jirangon, H.; Syed, M.A.; Shukor, M.Y. Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A. rzi. *BioMed Res. Int.* **2013**, *2013*. [CrossRef]

26. Underwood, E.J. *Trace Elements in Human and Animal Nutrition*; Academic Press: New York, NY, USA, 1971; pp. 356–357.
27. Kubota, J. Areas of molybdenum toxicity to grazing animals in the western states. *J. Range Manag.* **1975**, *28*, 252–256. [CrossRef]
28. Novotny, J.A.; Peterson, C.A. Molybdenum. *Adv. Nutr.* **2018**, *9*, 272–273. [CrossRef]
29. Jin, Q.; Kirk, M.F. pH as a primary control in environmental microbiology: 1. Thermodynamic perspective. *Front. Environ. Sci.* **2018**, *6*, 21. [CrossRef]
30. Glenn, J.L.; Crane, F.L. Studies on metalloflavoproteins: V. The action of silicomolybdate in the reduction of cytochrome c by aldehyde oxidase. *Biochim. Biophys. Acta* **1956**, *22*, 111–115. [CrossRef]
31. Krishnan, C.V.; Garnett, M.; Chu, B. Influence of pH and acetate on the self-assembly process of $(NH_4)_{42}[Mo^{VI}{}_{72}Mo^{V}{}_{60}O_{372}(CH_3COO)_{30}(H2O)_{72}]$.ca.300$H_2O$. *Int. J. Electrochem. Sci.* **2008**, *3*, 1299–1315.
32. Khayat, M.E.; Rahman, M.F.A.; Shukor, M.S.; Ahmad, S.A.; Shamaan, N.A.; Shukor, M.Y. Characterization of a molybdenum-reducing *Bacillus* sp. strain khayat with the ability to grow on SDS and diesel. *Rend. Fis. Acc. Lincei.* **2016**, *27*, 547–556. [CrossRef]
33. Roslee, A.F.A.; Gomez-Fuentes, C.; Zakaria, N.N.; Shaharuddin, N.A.; Zulkharnain, A.; Abdul Khalil, K.; Convey, P.; Ahmad, S.A. Growth optimisation and kinetic profiling of diesel biodegradation by a cold-adapted microbial consortium isolated from Trinity Peninsula, Antarctica. *Biology* **2021**, *10*, 493. [CrossRef] [PubMed]
34. Zahri, K.N.M.; Zulkharnain, A.; Gomez-Fuentes, C.; Sabri, S.; Abdul Khalil, K.; Convey, P.; Ahmad, S.A. The use of response surface methodology as a statistical tool for the optimisation of waste and pure canola oil biodegradation by Antarctic soil bacteria. *Life* **2021**, *11*, 456. [CrossRef] [PubMed]
35. Zakaria, N.N.; Gomez-Fuentes, C.; Abdul Khalil, K.; Convey, P.; Roslee, A.F.A.; Zulkharnain, A.; Sabri, S.; Shaharuddin, N.A.; Cárdenas, L.; Ahmad, S.A. Statistical optimisation of diesel biodegradation at low temperatures by an Antarctic marine bacterial consortium isolated from non-contaminated seawater. *Microorganisms* **2021**, *9*, 1213. [CrossRef] [PubMed]
36. Lee, G.L.Y.; Zakaria, N.N.; Convey, P.; Futamata, H.; Zulkharnain, A.; Suzuki, K.; Khalil, K.A.; Shaharuddin, N.A.; Alias, S.A.; González-Rocha, G.; et al. Statistical optimisation of phenol degradation and pathway identification through whole genome sequencing of the cold-adapted Antarctic bacterium, *Rhodococcus* sp. strain AQ5-07. *Int. J. Mol. Sci.* **2020**, *21*, 9363. [CrossRef]
37. Yakasai, H.M.; Karamba, K.I.; Yasid, N.A.; Halmi, M.I.E.; Rahman, M.F.; Ahmad, S.A.; Shukor, M.Y. Response surface-based optimization of a novel molybdenum-reducing cyanide-degrading *Serratia* sp. strain HMY1. *Desalin. Water Treat.* **2019**, *145*, 220–231. [CrossRef]
38. Yusuf, I.; Ahmad, S.A.; Phang, L.Y.; Syed, M.A.; Shamaan, N.A.; Abdul Khalil, K.; Dahalan, F.A.; Shukor, M.Y. Keratinase production and biodegradation of polluted secondary chicken feather wastes by a newly isolated multi heavy metal tolerant bacterium-*Alcaligenes* sp. AQ05-001. *J. Environ. Manag.* **2016**, *183*, 182–195. [CrossRef] [PubMed]