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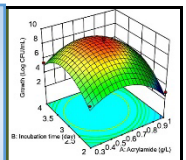

A Two-Level Factorial Design for Screening Factors that Influence the Growth of *Pseudomonas* sp. Strain Dry135 on Acrylamide

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ABSTRACT

Polyacrylamide is one of the most important sources of acrylamide in soil because it degrades into acrylamide over time. The breakdown of acrylamide by bacteria has experienced a steady but consistent increase in interest all over the world as a bioremediation technique. In this investigation, a previously obtained molybdenum-reducing bacterium with amide-degrading capabilities was found on critical parameters leading to optimum growth on acrylamide utilizing a two-level factorial design. The two-level factorial design was used in the screening of five independent parameters impacting the bacterium's growth on acrylamide. These variables include pH, temperature, incubation period, acrylamide concentration, and ammonium sulphate concentration. The two-factor factorial design was successful in identifying major contributing parameters in the growth of this bacterium on acrylamide, which were acrylamide concentration, pH, and incubation time ($p<0.05$), which can be further optimized using RSM in future research. ANOVA, Pareto's chart, pertubation's plot, and other diagnostic plots were used to analyze the significant contributing components or parameters. Diagnostic plots such as half-normal, Cook's distance, residual vs runs, leverage vs runs, Box-Cox, DFFITS, and DFBETAS all supported the two-level factorial result. The acrylamide range used in this study was well within the range reported to being tolerated by the majority of acrylamide-degrading bacteria. Incubation time is an expected finding because longer incubation time allows for higher growth, and incubation time ranging from two to five days for optimized growth has been documented in numerous acrylamide-degrading bacteria. Most acrylamide-degrading microorganisms grow well in near-neutral environments, and the results obtained in this investigation are consistent with published literature trends.

INTRODUCTION

Exposure to acrylamide in experimental animals has been linked to an increased risk of cancer [1, 2], and now evidence linking exposure to cancer in humans is emerging. Exposure to acrylamide increases the incidence of perinatal death, mutagenicity, clastogenicity, endocrine-related malignancies, and male reproductive toxicity in rats, according to studies [3]. Acrylamide has a negative effect on male rat reproductive systems because it produces histological abnormalities in the seminiferous tubules. The chemical is responsible for these histopathological abnormalities. Acrylamide can trigger a burning sensation or a rash if it is inhaled or absorbed via the skin. These two explanations are equally reasonable. Something is amiss with the neurological system if you have tingling in your tongue, excessive sweating, and general lethargy [1]. According to Yang et al. [4], *Salmonella* TA100 and TA98 strains exposed to acrylamide are susceptible to developing mutations as a result of the chemical. Mice that were injected intraperitoneally with acrylamide at a dose of 50 mg/kg showed an increase in

chromosomal abnormalities in their bone marrow after receiving the drug. To test the efficacy of the medicine, an injection was administered to the mice. Collections of lymphocytes from mice given intraperitoneal dosages of acrylamide up to 125 mg/kg showed no significant increase in the occurrence of chromosomal aberrations. This indicates that lymphocytes were not exposed to acrylamide via the digestive system. Intraperitoneal injection of acrylamide produced the observed effect [5].

The Maillard reaction is a chemical process that can take place when carbohydrate-rich meals are cooked at high temperatures. Acrylamide, a carcinogenic and neurotoxic molecule, may be produced in this reaction. Some foods, especially those that are heavy in carbohydrates, can generate acrylamide through the Maillard reaction. The food falls into this category. The Maillard reaction is a chemical reaction that takes place when sugars and amino acids are combined in just the right amounts. The production of acrylamide requires a series of steps, of which this is the first [6]. However, acrylamide can be produced from a wide range of carbonyl compounds [7]. In Sweden and Norway, acrylamide contamination in water sources led to the deaths of cattle and fish. Polyacrylamide (PAM), a byproduct of acrylamide synthesis, is widely employed in a variety of industries, including those involved in making adhesives, plastics, and printed materials, and even in the purification of drinking water. As of 2005, acrylamide, a potentially dangerous monomer, was found in most commercial polyacrylamides. As a direct result of polyacrylamides being widely available for commercial use, this condition has had a considerable impact on our food supply chain. The herbicide Roundup, the source of acrylamide in farmland, contains a concentration of 30 percent polyacrylamide. This problem, which needs fixing, can only be fixed by resolving the acylamide in the environment by a biological mechanism [8].

Because of its high water solubility, acrylamide can cross the skin, lungs, digestive tract, and even the placental barrier and enter the body. The fact that it dissolves in water contributes to its malleability. The amount of acrylamide adducts in haemoglobin can be used to estimate the amount of acrylamide that the general public is exposed to as a result of their occupations. The results showed that 41 workers in an acrylamide manufacturing plant had levels of neurotoxicity measured by haemoglobin adducts. In the Chinese acrylamide factory, workers were exposed to dangerously high levels of the chemical, as evidenced by a rise in the number of adducts formed on their haemoglobin [9]. Multiple cases of acute acrylamide poisoning have been documented in Japan as a result of the contamination of the country's water supply with acrylamide. The grouting procedure fouled a well at a depth of 2.5 meters, with Igisu et al. [10] reporting an acrylamide level as high as 400 mg acrylamide/L. The testing of the well led to this discovery. The study found that five patients who drank the acrylamide-tainted water experienced symptoms like truncal ataxia and disorientation.

Polluted air or food or drink can introduce acrylamide into the body. Once it reaches the skin, mucous membranes in the lungs, or the digestive tract, it is absorbed into the body. Conversely, it will be eliminated via the urinary system after being digested [11-13]. The impact of acrylamide is aided by the fact that it is present in biological fluids and can travel to various parts of the body. Acrylamide has been detected in plasma and other bodily fluids. Due to its high reactivity against proteins, acrylamide still poses a risk to people and workers even if it is quickly digested and eliminated after exposure. This holds true despite the fact that it is rapidly absorbed and eliminated after being exposed. Soil contamination is especially concerning, and this has motivated scientists to research ways to eliminate acrylamide. It is difficult, if not impossible, to remove acrylamide from soils because of the soil's complex matrix. Attractive as a means of breakdown and cleanup is the use of microorganisms, which, under aerobic circumstances, may completely convert acrylamide to non-hazardous water and carbon dioxide through their metabolism. Although other microorganisms, such as the yeast *Rhodotorula* sp. [14] and the fungus *Aspergillus oryzae* [15], have been characterized as capable of degrading acrylamide, bacteria continue to be the most regularly reported acrylamide degraders [16-25]. Tuning the regulating parameters is essential for acrylamide growth research.

When conducting basic research, scientists often rely on a "intuitive" approach when arranging experiments. The "one factor at a time" method has long been used in biology research (OFAT). In this method, the only variable is the researched entity itself, and all other factors are held constant. This method may have the ability to discover enormous "major impacts" in biological research, but it will lead to erroneous statements due to interactions between components. Regulating a large number of input factors is crucial for optimal results due to the intricacy of the process. Results from an experiment may be chaotic, but there may be a wealth of new information to go through. This allows for a more interesting data set to be collected by adjusting the data point selection in accordance with a statistically sound experimental design. The DOE's foundational problem structure accounts for a wide range of variables that are assumed to affect product quality. Which of several feasible layouts for an experiment yields the most anticipated data is chosen as the final layout. The precision or accuracy with which the fitted model estimates the input variable or forecasts the output variable is often used to establish this criterion. The nature of this partnership is rarely understood. Although several studies have used OFAT to optimize processes and increase responsiveness, optimizing increasingly complicated procedures will require an understanding of the interdependencies between their constituent parts. An OFAT method involves optimizing a single axis before moving on to the next. If the study's initial premise makes sense, finding the global maximum that maximizes the output variable should be possible. One important consideration is that the search may have been terminated at a local maximum or pseudo-optimum.

In the early stages of testing, when in-depth system knowledge is typically lacking, the Plackett-Burman (PB) experimental design is a popular screening tool for identifying crucial components. The names of its creators, Plackett and Burman, are associated with this strategy. In 1946, statisticians Robin L. Plackett and J.P. Burman developed it to find causal factors with minimal testing. Two-factor interactions can have substantial effects when employing a Plackett-Burman design. When there is very little chance of two-way contact, these kinds of designs are appropriate. Because it is the smallest design, not enough data has been collected to know what the effects of the factors are in two-level multi-factor studies with more than four variables, but the Plackett-Burman design is effective for discovering big main effects. The two-level factorial design surpasses the PB approach in the screening process because it takes into account the interactions between the many factors. This method not only computes the connections between significant cultural aspects but also provides a more accurate assessment of the optimal condition. Two-level factorial design has been used to improve the efficiency of many different types of screening procedures [26,26-31]. Here we describe the use of

a two-level factorial design to screen for significant factors that influence the growth of *Pseudomonas* sp strain DrY135 on acrylamide.

MATERIALS AND METHODS

In the early stages of testing, when in-depth system knowledge is usually missing, the Plackett-Burman (PB) experimental design is a popular screening approach used to discover important components. The approach was coined by Plackett and Burman. To find causal factors using as few tests as possible, statisticians Robin L. Plackett and J.P. Burman invented it in 1946. In a Plackett-Burman experiment, two-factor interactions may obscure the significance of the design's main effects. Designing in this manner is appropriate when there is very little chance of a two-way exchange of information taking place. In two-level multi-factor experiments with more than four factors, the Plackett-Burman design is useful for detecting large main effects; however, PB does not verify if one factor's effect depends on another, and because it is the smallest design, not enough data has been collected to know what those effects are. When it comes to the screening phase, the two-level factorial design is preferable to the PB technique since it takes into account the interplay between the various components. This approach yields a more precise estimate of the optimum situation and computes the interdependencies of major cultural elements. There are several examples of two-level factorial design being useful in screening processes throughout the literature [26,26-31].

Growth and maintenance of acrylamide-degrading bacterium

Prior to this discovery, the bacterium was identified as a Mo-reducer [32]. The bacterium was grown in nutrient broth overnight, and then 0.1 mL was added to 45 mL of acrylamide enrichment medium in a 100 mL volumetric flask. The flask was placed in an incubator shaker (Certomat R, USA) set to 25 °C and 150 rpm, and the culture was allowed to grow for 48 hours. The bacterium was isolated in minimal salt medium (MSM), which included 0.5 g acrylamide g/L, glucose 10 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 6.8 g/L, $FeSO_4 \cdot H_2O$ 0.005 g/L and 10 mL of H_3BO_3 0.05 g/mL, $ZnCl_2$ 0.03 g/L, $CoCl_2 \cdot 6H_2O$ 0.003 g/mL, $Cu(CH_3COO)_2 \cdot H_2O$ 0.01g 0.002 g of $FeCl_2 \cdot 6H_2O$. pH was changed to 7.5 or to the required pH using HCl or NaOH, but within the buffering species of phosphate already present in the medium.

Screening of significant parameters using two level factorial design

Despite the presence of complex interactions, the two-level factorial design was used for the primary goal of identifying the relative importance of a large number of independent variables. As there were five variables involved, we used a 2-factorial design. Bacterial proliferation was the result, as measured by the log CFU/mL scale. All of the tests were organized and performed in the order shown in **Table 1**. Each experiment was repeated twice, and the averages and standard deviations are presented below. One piece of software (Design Expert 7.0, developed by Stat-Ease, Inc.) was used to analyze the data and identify which of these parameters is more crucial than the others (trial version).

Statistical Analysis

The values are presented as means standard deviations (three replicates). Between-group comparisons were made using either one-way analysis of variance (with post hoc analysis by Tukey's test) or the Student's t-test. Any value below 0.05 was judged to be non-trivial. Values will be rounded up or down to three decimal places as needed.

Table 1. Coded and actual range for the regular two- level factorial analysis.

Factor	Name	Units	Min-imum	Max-imum	Coded Low	Coded High	Mean	Std. Dev.
A	pH		5.80	7.80	-1 ↔ 5.80	+1 ↔ 7.80	6.80	1.03
B	Temperature	oC	20.0	40.0	-1 ↔ 20.00	+1 ↔ 40.0	30.0	10.33
C	Acrylamide	g/L	0.10	1.0	-1 ↔ 0.10	+1 ↔ 1.00	0.55	0.4648
D	Amm SO4	g/L	1.0	10.0	-1 ↔ 1.00	+1 ↔ 10.0	5.5	4.65
E	Incubation time	Days	1	4	-1 ↔ 1.00	+1 ↔ 4	2.5	1.55

RESULTS

Two-level factorial design for screening the operational factors

There were five operational parameters investigated for the factor screening investigation, including pH, temperature, incubation duration, acrylamide concentration, and ammonium sulphate concentration, in a standard two-level factorial layout. The bacteriuml growth rate was found to be 7.64 log CFU/mL to 9.64 log CFU/mL, between a minimum and maximum range of 0 and 100. The design plan is depicted in **Table 1**, which lists the values of the experiment's variables, as well as the values of the experiment itself and the predicted values of the response variables.

Table 2 displays the results of an F-test, an ANOVA, and the P-value. The model's statistical validity is determined by these analyses. The results demonstrated that the model is statistically significant, with a F value of 38.64 and a P value of less than 0.0001. That the model has a little P value indicates this. The reliability of the model can be determined by computing the correlation coefficient (R^2: 0.930, which is near unity) and the adjusted correlation coefficient (Adj R^2: 0.905), which indicates that 90% of the total variance in response data is explained. The adequacy accuracy was calculated to be 17.68, which means the model produces a usable signal for navigating the design area. Additionally, P-values 0.05 confirm the importance of model components, and in this case, terms A, C, E, and the interaction AE were all significant.

Table 2. Analysis of variance (ANOVA) for regular two- level factorial analysis.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.26	4	1.07	36.68	< 0.0001	significant
A-pH	0.4661	1	0.4661	16.04	0.0021	
C-Acrylamide	0.8657	1	0.8657	29.80	0.0002	
E-Incubation time	2.84	1	2.84	97.68	< 0.0001	
AE	0.0932	1	0.0932	3.21	0.1009	
Residual	0.3196	11	0.0291			
Cor Total	4.58	15				
Fit statistics						
Std. Dev.	0.1705		R^2	0.9303		
Mean	8.60		Adjusted R^2	0.9049		
C.V. %	1.98		Predicted R^2	0.8524		
			Adeq Precision	17.3048		

By applying two-factor interactive method, the predicted bacterial growth as the response can be obtained and given in terms of coded and actual factors equation (**Table 3**).

Table 4. Coded and actual predicted values for the for two-level factorial design.

Coded Growth	Factor	Actual Growth	Factor
+8.60		+7.89099	
+0.1707	A	+0.043512	pH
-0.2326	C	-0.516906	Acrylamide
+0.4212	E	-0.065133	Incubation time
+0.0763	AE	+0.050868	pH * Incubation time

Standard errors, confidence intervals, and variance inflation factors for the computed coefficients of the components under study are listed in **Table 4** (VIF). Only incubation time and pH show positive coefficients among the chosen components, with incubation time producing a larger positive value than pH. It appears that the incubation time has a greater influence on the growth of these bacteria on acrylamide than the other parameter. However, a negative value is revealed by the coefficient estimate of the acrylamide concentration, indicating that this bacterium's development when fed acrylamide is stunted at concentrations over the optimum. How much the variance of a given model coefficient is inflated due to a lack of orthogonality in the design can be calculated with a statistic called the variance inflation factor (VIF). For a given model coefficient, the square root of the VIF determines how much larger the standard error is for the VIF design compared to the orthogonal design.

A VIF of 1 is preferred above other values since it denotes that the coefficient is orthogonal to the other model components (the correlation coefficient is zero). In contrast, VIFs above 10 may signal cause for concern. Furthermore, VIFs greater than 100 are concerning because they suggest that coefficients were estimated wrongly due to multicollinearity, and VIFs greater than 1000 are the result of severe collinearity. The multicollinearity in the regression study was confirmed by finding the variance inflation factor (VIF) to be 1. Two-level factor analysis revealed that only three of the five screening parameters were significant.

Table 4. Coefficient estimate obtained during ANOVA for two-level factorial design.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	8.60	1	0.0426	8.51	8.70	
A-pH	0.1707	1	0.0426	0.0769	0.2645	1.0000
C-Acrylamide	-0.2326	1	0.0426	-0.3264	-0.1388	1.0000
E-Incubation time	0.4212	1	0.0426	0.3274	0.5149	1.0000
AE	0.0763	1	0.0426	-0.0175	0.1701	1.0000

It is obvious from the Pareto charts that were created for the study of each response coefficient for its statistical significance and which are displayed in **Fig. 1**. Bonferroni limit line (t-value of effect: 3.728) and t-limit line are the names of the two limit lines that are used in the Pareto chart to categorize the t-value of the effect (t-value of effect: 2.201). There are three distinct categories for determining the importance of coefficients. The first coefficient to have a t-value of effect that is higher than the limit set by Bonferroni is the one that is regarded as significant. The second coefficient with a t-value of effect that falls between the Bonferroni line and the t-limit line is referred to as coefficients likely to be significant, and the third coefficient with a t-value of effect that falls below the t-limit line is a statistically insignificant coefficient that could be removed from the analysis. Both of these coefficients have t-values that are between the Bonferroni line and the t-limit line. In a Pareto chart, the results indicate that incubation period and pH are the positively influencing factors, while acrylamide concentration is shown to be a significant contributor, but in a negatively interpreting way. These findings are similar to what was found when using the coefficient estimate.

The acrylamide concentration, pH, and incubation duration were the main contributing parameters in the cellular growth of this bacterium on acrylamide. These are characteristics that have been discovered in numerous OFAT-based approaches as being crucial in contributing high growth of microorganisms on acrylamide. This work was carried out using acrylamide concentrations that were well within the range that was known to be tolerated by the majority of microorganisms capable of acrylamide degradation. Acrylamide concentrations that are greater than 1000 mg/L are normally harmful to microorganisms [16–25]. It is clear from the Pareto charts that were created for the investigation of each response coefficient's statistical significance (**Fig. 1**).

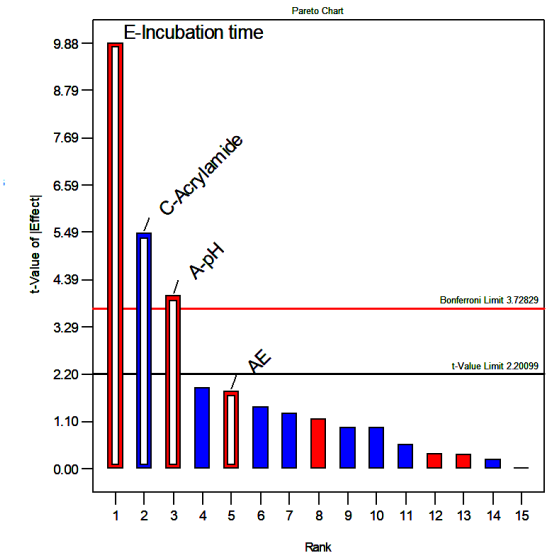

Fig. 1. Pareto chart of operational parameters.

The t-value of the effect is separated into two categories in the Pareto chart: the Bonferroni limit line (t-value of effect: 3.728), and the t-limit line (t-value of effect: 2.201). The relevance of coefficients may be broken down into three distinct areas. It is accepted that a coefficient is significant if it is the first one to have a t-value of effect that is greater than the limit set by Bonferroni. The second coefficient with a t-value of effect that falls between the Bonferroni line and the t-limit line is referred to as coefficients likely to be significant, and the third coefficient with a t-value of effect that falls below the t-limit line is a statistically insignificant coefficient that could be removed from the analysis. Both of these coefficients have t-values that fall between the Bonferroni line and the t-limit line. In the Pareto chart, the findings reveal that incubation duration and pH are the components that have a positive influence, whereas acrylamide concentration is a substantial contribution but in a way that has a negative impact. This is similar to what was found with the coefficient estimate results.

The important contributing parameters in the growth of this bacterium on acrylamide were the acrylamide concentration, the pH, and the incubation time. These are factors that have been identified in several OFAT-based approaches as being important in contributing high growth of microorganisms on acrylamide. This investigation was conducted out using acrylamide concentrations that fell well within the range that previous research has shown to be tolerated by the majority of bacteria capable of acrylamide degradation. Acrylamide concentrations of more than 1000 mg/L are normally harmful to microorganisms.

The perturbation plot (**Fig. 2**) of the model exhibits the comparative effect of all the operational parameters at a particular point in the design space. From the plot, it can be observed that factor E (incubation time) has the highest slope, followed by factor C (acrylamide) and A (pH), which indicates factor's E highest sensitivity to the response. The perturbation plot reveals the presence of interaction between pH and incubation period, a feat that the Plackett-Burman screening method would not be able to detect [36–39].

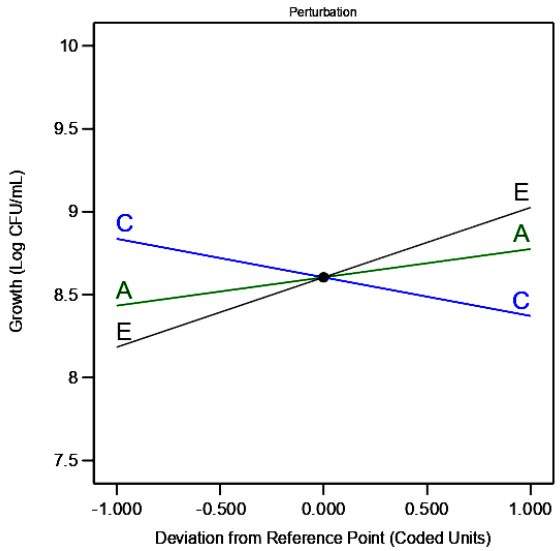

Fig. 2. Perturbation plot of operational parameters obtained through regular two-factor design.

To check the validity of the normality assumption, we constructed and analyzed a half-normal probability plot of the residuals (**Fig. 3**). If all of the internally studentized residuals values are less than 2 and lie on a straight line, then there is no need to transform the response. **Fig. 4** shows that the values predicted by the model agree quite well with the actual experimental data. **Fig. 5** shows a Box-Cox plot, which may be used to determine the optimal power law transformation given a given value of lambda.

It is not recommended to further change the observed response in order to match the model because the 95 percent confidence interval has a value of 1 that corresponds to the value that was designed into the model. Each of the obtained values is within the standard range of 0–1 as seen in **Fig. 6** of the leverages vs. run plot. As a result, it's possible that one of the design points will affect the model's accuracy. A high leverage point value over one is deemed "bad" if there is a problem with the data point, like an unexpected error, because the error has a large effect on the model.

Looking at the chart of leverages against runs, it was noticed that there are no points above the mean leverage. This is because any points above the mean leverage would have an effect on at least one model parameter. The scatter plot of Cook's distances can be used to produce a measure of the response outlier that is analogous to a single experimental trial (**Fig. 7**). When evaluating the significance of an observation, a higher Cook's distance is preferred because it cannot be negative. If Cook's D is three times the dataset's mean value, then the finding is regarded as significant by most researchers. According to this research, all of the calculated Cook's distances fall inside a uniform range from 0 to 1. The plot of the residuals against the run data (**Fig. 8**) does not reveal any signs of serial correlation, which leads one to believe that the data is random in terms of its features [26,27,30,40,41].

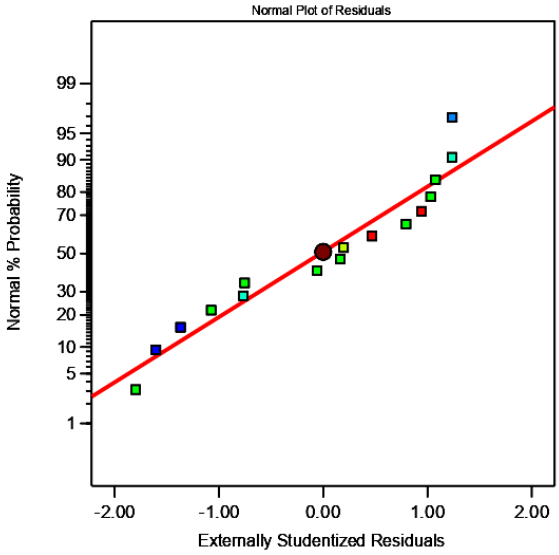

Fig. 3. Diagnostic's plot in the form of the normal plot of residuals for the two-level factorial optimization studies.

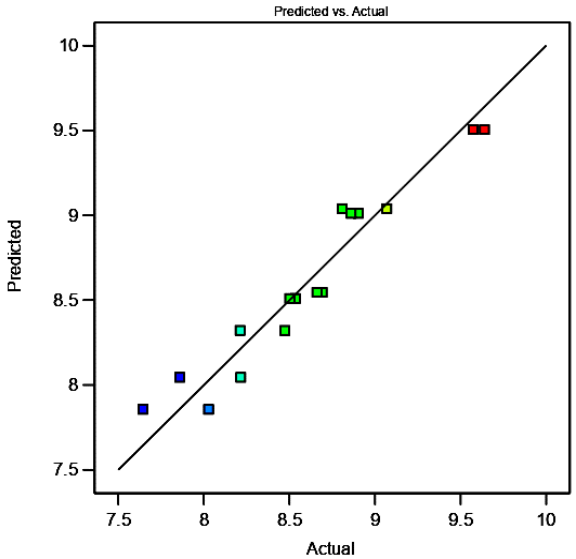

Fig. 4. Diagnostic's plot in the form of the predicted versus actual plot for the two-level factorial optimization studies.

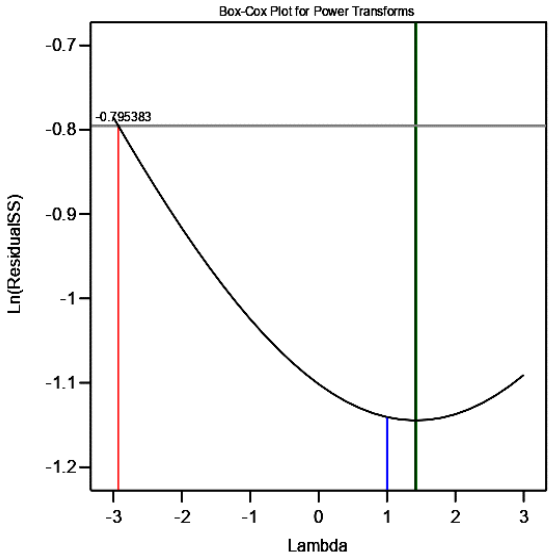

Fig. 5. Diagnostic's plot in the form of Box-Cox plot for the two level factorial optimization studies.

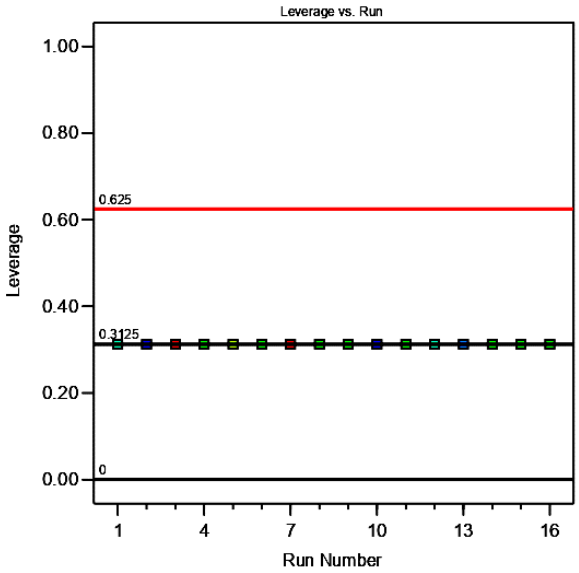

Fig. 6. Diagnostic's plot in the form of leverage vs runs for the two-level factorial optimization studies.

It's not always a problem when influential points are brought up, but it is important to follow up on observations that are marked as extremely influential. A high result on an influence measure may indicate a number of different things, including a mistake in the data input process or an observation that is clearly not typical of the population of interest and so need to be excluded from the analysis. During the process of fitting a model, the inclusion of one or more data points that are sufficiently important might cause coefficient estimations to be thrown off and muddle the model's interpretation. In the past, before conducting a linear regression, the potential of outliers in a dataset would be evaluated using histograms and scatterplots. This was done before running the linear regression. Both approaches of evaluating data points were subjective, and there was little way to determine how much influence each possible outlier had on the data representing the outcomes.

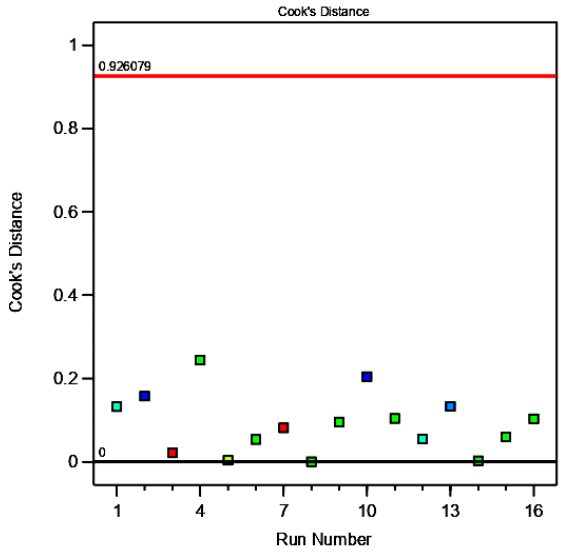

Fig. 7. Diagnostic's plot in the form of Cook's distance vs runs for the two level factorial optimization studies.

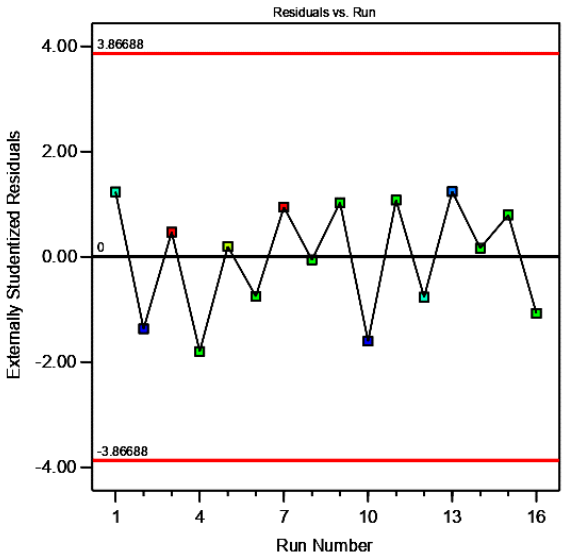

Fig. 8. Diagnostic's plot in the form of residuals vs runs for the two-level factorial optimization studies.

This resulted in the development of a number of quantitative metrics, such as DFFIT and DFBETA. The DFFFITS algorithm assesses how much of an impact each particular example has on the value that was anticipated. It is possible to translate it to the distance according to Cook. DFFITS, in contrast to Cook's distances, can take either a positive or a negative value. When the value is "0," the point in question is located precisely on the regression line. Leverage is what makes this possible. Mathematically speaking, it is the difference between the expected value with observation and the predicted value without observation. According to the alternative formula, DFFITS is the externally studentized residual (ti) with strong leverage points multiplied by it and low leverage points reducing it [39,42,43]. The plots show the DFBETAS values (**Fig. 9**) were within the size-adsjuted threshold acceptable range while the DFFITS values were within the cut-off values (**Fig. 10**).

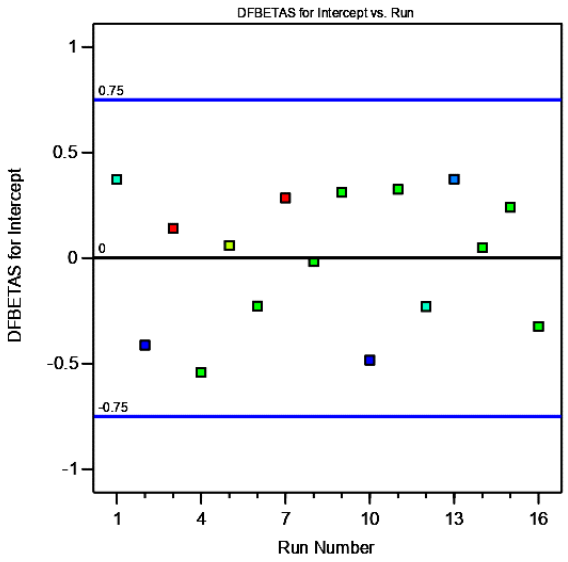

Fig. 9. Diagnostic's plot in the form of DFBETAS for intercept vs runs for the two level factorial optimization studies.

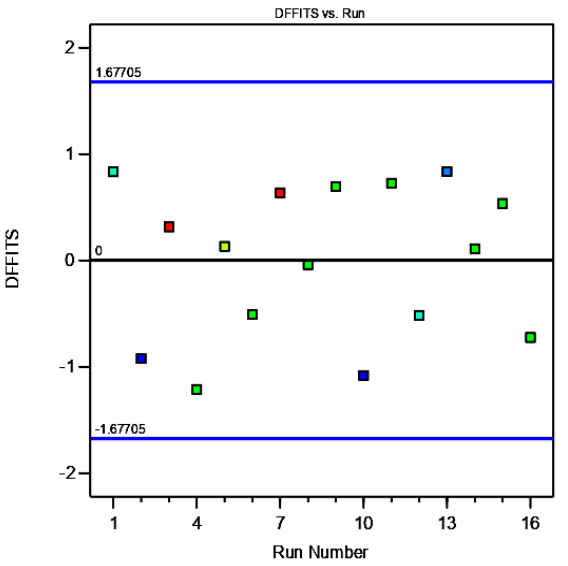

Fig. 10. Diagnostic's plot in the form of DFFITS vs runs for the two level factorial optimization studies.

CONCLUSION

It was determined that there were five distinct parameters that affected bacterial growth when exposed to acrylamide, thus a two-level factorial design was used to test their effects. Among these are the levels of ammonium sulfate, acrylamide, pH, and incubation duration. With the help of a two-factor factorial design, we were able to identify the acrylamide concentration, pH, and incubation duration as significant contributors to the cellular growth of this bacterium on acrylamide, all of which can be adjusted further with RSM in subsequent studies. We used analysis of variance, Pareto charts, pertubation plots, and other diagnostic plots to determine which factors and parameters were most influential. Half-normal, Cook's distance, residual vs. runs, leverage vs. runs, Box-Cox, DFFITS, and DFBETAS diagnostic plots all corroborated the two-level factorial conclusion. The concentrations of acrylamide used in this study were within the range previously reported to be tolerated by the vast majority of microorganisms capable of digesting acrylamide. The longer the incubation period, the more growth will occur, therefore this is to be expected. In many acrylamide-degrading bacteria, incubation periods of two to five days have been found to achieve optimal development. The results obtained in this investigation are consistent with the general trend in the published literature that most of acrylamide-degrading microorganisms thrive under near-neutral environments.

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