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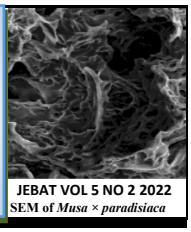

A Two-Level Factorial Design for Screening Factors that Influence the Growth of *Bacillus* sp. Strain ZEID-14 on Acrylamide

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ABSTRACT

Acrylamide is often used to strengthen soil structure and usually leachate from this application forms a major source of acrylamide pollution in the environment. The soil matrix means physicochemical methods of removal will be costly if not difficult. Bioremediation using acrylamide-degrading bacteria is an appealing technique. In this investigation, a previously isolated molybdenum-reducing bacterium with the ability to degrade amides was discovered based on critical characteristics contributing to optimal growth on acrylamide utilizing a two-level factorial design. Five independent parameters that influence the growth of the bacterium on acrylamide were evaluated using a two-level factorial design. Among these variables are pH, temperature, incubation period, acrylamide content, and glucose concentration. The two-factor factorial design was successful in identifying significant contributing parameters to the growth of this bacterium on acrylamide, namely acrylamide concentration, pH, and incubation time, which can be further otpimized using RSM in future studies. Using ANOVA, Pareto's chart, perturbation's plot, and other diagnostic plots, the significant contributing factors or parameters were examined. Half-normal, Cook's distance, residual vs runs, leverage versus runs, Box-Cox, DFFITS, and DFBETAS diagnostic plots all supported the two-level factorial result. This work was conducted using acrylamide concentrations well within the known tolerance range of most acrylamide-degrading bacteria. Incubation time is an expected consequence, as longer incubation time permits more growth. The majority of acrylamide-degrading microorganisms thrive under near-neutral circumstances, as indicated by the results of our investigation, which are consistent with previous literature trends.

INTRODUCTION

Despite the fact that it has been demonstrated that exposure to acrylamide may result in the development of cancer in experimental animals [1], evidence in humans who have been exposed to the chemical is beginning to emerge [2]. There is an association between acrylamide exposure and an increased risk of perinatal death, mutagenicity, clastogenicity, endocrine-related cancers, and male reproductive toxicity in rats, according to the findings of relevant studies [3]. Because acrylamide causes histological abnormalities in the seminiferous tubules, this chemical also has a deleterious effect on the reproductive systems of male rats. The chemical is responsible for these histological abnormalities. If acrylamide is inhaled or absorbed through the skin, the user may experience a burning sensation or develop a rash. Both of these responses are plausible. A tingling tongue, an overactive sweat gland, and a sluggish body are all signs of a problem with the nervous system [1]. According to Yang et al. [4], *Salmonella strains* TA100 and TA98 that have been exposed to acrylamide are sensitive to mutagenesis as a result of this chemical exposure. A greater number of chromosomal abnormalities were identified in the bone marrow of mice that had received an intraperitoneal injection of 50 mg/kg acrylamide after receiving the drug. The mice were injected with the

medication in order to evaluate its effects. The incidence of chromosomal abnormalities in lymphocytes collected from mice given up to 125 mg/kg of acrylamide intraperitoneally did not rise considerably when acrylamide was administered in this manner. This suggests that acrylamide did not enter the lymphocytes via the digestive system. This effect was seen following intraperitoneal administration of acrylamide [5].

When carbohydrate-rich foods are cooked at a high temperature, a chemical reaction known as the Maillard reaction may occur. This interaction could result in the formation of acrylamide, a chemical capable of causing cancer as well as damage to the neurological system. The Maillard process has the ability to produce acrylamide in certain foods, especially those with a high carbohydrate content. These are the various forms of food. When sugars and amino acids are mixed in the proper proportions, a chemical reaction known as the Maillard reaction occurs. This is the first stage in a sequence of reactions that will eventually result in the formation of acrylamide [6]. Alternatively, acrylamide can be synthesized from a variety of different carbonyl compounds [7].

Both cattle and fish perished in Sweden and Norway as a direct result of acrylamide contamination in nearby waterways. In the production of adhesives, plastics, and printed materials, as well as in the treatment of drinking water, polyacrylamide, abbreviated as PAM, is the most prevalent use of acrylamide. As of 2005, commercial polyacrylamides are regularly contaminated with the hazardous acrylamide monomer. Due to the extensive usage of these compounds and the commercial availability of polyacrylamides, this situation has had a tremendous influence on our food supply chain. The herbicide Roundup, which is responsible for the pollution of agricultural soil with acrylamides, may include a concentration of thirty percent polyacrylamide. The acrylamide in the environment must be remediated by a biological process [8] in order to handle this issue, which must be addressed in order to be resolved.

Due to its high water solubility, acrylamide can be absorbed through the skin, lungs, digestive system, and even the placental barrier. Its dissolvability in water confers this adaptability. By examining the amount of acrylamide adducts in haemoglobin, it is possible to compute the amount of acrylamide that the general public is exposed to as a result of their jobs. According to the data, the biomarker haemoglobin adducts were associated with neurotoxicity in 41 personnel of an acrylamide manufacturing facility. In the Chinese facility that manufactures acrylamide, the level of haemoglobin adducts increased, indicating that the workers were exposed to extremely high quantities of acrylamide [9]. As a result of acrylamide contamination in the country's water system, multiple cases of acute acrylamide poisoning have been reported in Japan. Igisu et al. [10] found that a well that had been contaminated by a grouting operation at a depth of 2.5 meters had as much as 400 mg acrylamide/L. This discovery was made after testing the well. Five patients who drank acrylamide-tainted drinking water exhibited symptoms like truncal ataxia and confusion, according to the study.

Acrylamide enters the body through inhaling contaminated air or swallowing or drinking something that is tainted in some way. It is then absorbed by the mucous membranes of the lungs, digestive tract, or skin. Once it has been digested, however, it will be flushed out of the body [11–13]. The presence of acrylamide in biological fluids and its distribution throughout the body both contribute to the enhancement of acrylamide's effect. Acrylamide is prevalent in bodily fluids. Despite being rapidly digested and removed after exposure, acrylamide poses a threat to individuals and employees due to its high protein reactivity. This is the case despite the fact that it is rapidly digested and removed following exposure. Researchers have been encouraged to find strategies to reduce acrylamide, notably the soil contamination it causes. Nevertheless, acrylamide remediation in soils is difficult, if not impossible, due to the soil's complex matrix. The use of microorganisms in the decomposition and cleaning up of acrylamide is advantageous because, under aerobic conditions, the metabolism of microorganisms facilitates the complete conversion of acrylamide to non-hazardous water and carbon dioxide. Microorganisms with acrylamide-degrading and absorbing capabilities have been described in the scientific literature, including the yeast *Rhodotorula* sp. [14] and the fungus *Aspergillus oryzae* [15], however, bacteria remain the most often reported acrylamide degraders [16–25]. For acrylamide growth experiments, the regulating parameters must be tuned.

Experimentation planning in fundamental research is typically governed by "intuition." Biology experiments have always been conducted "one variable at a time" (OFAT). In this procedure, all factors and variables are held constant with the exception of the researched object, and the object's output is analyzed. This technique has the potential to reveal important "major impacts" in biological research, yet the interplay between its components will result in misspelt words. To get optimal results, it is necessary to regulate a high number of input elements due to the complexity of the process. Experiment outcomes may be noisy, and there may be a great deal of exciting incoming data. In such cases, the selection of data points can be modified to maximize the quantity of relevant information gathered through the use of a statistically-based experimental design, which can lead to much more interesting data.

The DOE's basic issue structure takes into account a variety of factors believed to influence process output. The experiment design that is finally chosen is determined by which of the numerous possible designs produces the greatest amount of anticipated data. Typically, this criterion is based on the precision or accuracy of the fitted model's estimates of the input variable or its projections of the output variable. The mechanics of this collaboration are typically unknown. Even if various studies on process optimization have utilized OFAT to improve responsiveness, it will be necessary to comprehend the interdependencies between components in order to optimize increasingly complex processes. Using the OFAT approach, one axis would be optimized before the other. If by some stroke of luck, the beginning of the study was reasonable, then it is possible to identify the global maximum that maximizes the output variable. Keep in mind, however, that there is a substantial chance that the search was halted at a local maximum or pseudo-optimum.

The Plackett-Burman (PB) experimental design is a well-known screening technique used to identify important system components during the early stages of experimentation when comprehensive system knowledge is generally missing. This technique was called after its inventors, Plackett and Burman. It was created by statisticians Robin L. Plackett and J.P. Burman in 1946 with the intention of identifying active variables with the fewest possible experiments. Using a Plackett-Burman design, two-factor interactions can obscure the significance of big effects. When there is little to no opportunity for two-way interaction, these types of layouts should be utilized. Although the Plackett-Burman design is useful for detecting large main effects in two-level multi-factor experiments with more than four factors, it does not verify whether one factor's effect is dependent

on another and because it is the smallest design, not enough data has been collected to determine what those effects are. In the screening phase, the two-level factorial design is superior to the PB technique because it takes into account how the different components interact with one another. This method yields a more precise evaluation of the best situation and assesses the interrelationships among major cultural elements. Numerous screening techniques have benefited from two-level factorial designs [26,26–31] in the literature. Here, we explain the use of a two-level factorial design to identify parameters that significantly affect the development of *Bacillus* sp. strain ZEID-14 on acrylamide.

MATERIALS AND METHODS

Even though they had been generated in large quantities, all of the chemical reagents were utilized in their unpurified stages during the inquiry. In addition, the analytical quality of all materials included in this investigation was maintained throughout the entire procedure. Experiments were conducted in triplicate in every instance unless otherwise specified in the study's accompanying notes.

Growth and maintenance of acrylamide-degrading bacterium

The bacterium was earlier isolated as a molybdenum-reducing bacterium from Sudanese soil [32]. On minimal salts medium (MSM) supplied with solely acrylamide as the nitrogen source and glucose as the only carbon source, this bacterium was characterized in relation to acrylamide. The bacteria was revived from a 16 percent glycerol stock by cultivating the pure culture overnight in 10 mL of nutrient broth. From this, 0.1 mL was added to 45 mL of acrylamide enrichment media in a 100 mL volumetric flask, and the culture was cultured on an incubator shaker at 25 °C for 48 hours at 150 rpm and 150 rpm (Certomat R, USA). Minimal salt medium (MSM) for growth was supplemented with 0.5 g acrylamide g/L as the sole nitrogen source, glucose 10 g/L as the carbon source, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 6.8 g/L (buffering species and source of phosphorous), $FeSO_4 \cdot H_2O$ 0.005 g/L and 0.1 mL of trace elements [8].

The presence of phosphate in the medium serves as a buffer, maintaining a pH range between 5.8 and 7.4. Acrylamide was the only nitrogen source utilized in the sterilisation procedure, and 0.45 micron-sized PTFE syringe filters were utilized. Samples of one millilitre each were sequentially diluted in sterile tap water and plated on nutrient agar to assess the number of bacteria present. The buffering effect of phosphate in the medium maintains a range of pH values between 5.8 and 7.8. Acrylamide was filtered-sterilized with 0.45 micron-sized PTFE syringe filters. To assess the quantity of bacteria present, repeated dilutions of one millilitre samples were performed. To assess the quantity of bacteria present, repeated dilutions of one millilitre samples in sterile tap water and overnight plating on nutrient agar were performed.

Screening of significant parameters using two level factorial design

Despite the presence of complex interactions, the two-level factorial design was applied to determine the relative relevance of a number of different elements that had an influence. We utilized a 2-factorial design with a total of five elements. The value represented by the code -1 was a lower value than the value indicated by the code 1. Using a log CFU/mL scale, bacterial growth was the observed response. The tests were designed and conducted in accordance with **Table 1**'s order. Each experiment was conducted twice, and the means of both sets of results are presented here. The data were processed by software (Design Expert 7.0, Stat-Ease, Inc.'s) in order to establish which of these parameters is significantly more essential than the others (trial version).

Table 1. Summary of factors and coded values.

Factor	Name	Units	Mini-mum	Max-imum	Coded Low	Coded High	Mean	Std. Dev.
A	pH		6.00	7.50	-1 ↔ 6.00	+1 ↔ 7.50	6.75	0.7746
B	Temperature	oC	20.0	40.0	-1 ↔ 20.00	+1 ↔ 40.00	30.00	10.33
C	Acrylamide	g/L	0.30	0.70	-1 ↔ 0.30	+1 ↔ 0.70	0.50	0.2066
D	Glucose	g/L	1.00	10.00	-1 ↔ 1.00	+1 ↔ 10.00	5.50	4.65
E	Incubation time	Days	2.00	4.00	-1 ↔ 2.00	+1 ↔ 4.00	3.00	1.03

Statistical Analysis

Values are means ± SD. In triplicate. One-way analysis of variance (with post hoc analysis by Tukey's test) or Student's t-test was used to compare between groups. P-value of < 0.05 was considered was significant. Whenever appropriate, values will be truncated to three decimal points.

RESULTS

Two-level factorial design for screening the operational factors

In factor screening study, five operational parameters (pH, temperature, incubation time, acrylamide concentration and glucose concentration) were considered for a regular two-level factorial design. Within the range of minimum and maximum values that were investigated, the bacterial growth rate ranged from 7.295 log CFU/mL to 9.159 log CFU/mL. **Table 1** provides an illustration of the design plan, which includes the actual values of the variables that were used in the experiment, as well as the experimental values, projected or predicted response values and residuals.

Table 1. Two-level factorial design for screening best parameters for the growth of *Bacillus* sp. strain Zeid-14.

Run	A: pH	B: Tempe-rature	C: Acry-lamide	D: Glucose	E: Incuba-tion time	Growth (Log CFU/mL)	Predicted growth (Log CFU/mL)	Residual
1	6	20	0.7	1	2	7.64	7.31	0.326
2	7.5	20	0.3	10	4	9.16	8.9	0.258
3	7.5	20	0.7	10	2	7.41	7.63	-0.226
4	6	20	0.7	10	4	8.16	8.1	0.062
5	6	40	0.3	10	4	8.75	8.58	0.170
6	6	20	0.3	1	4	8.27	8.58	-0.310
7	7.5	20	0.3	1	2	8.48	8.12	0.370
8	6	40	0.7	1	4	7.84	8.1	-0.263
9	6	20	0.3	10	2	7.55	7.79	-0.239
10	7.5	40	0.3	10	2	7.97	8.12	-0.144
11	6	40	0.7	10	2	7.64	7.31	0.327
12	7.5	40	0.7	1	2	7.29	7.63	-0.340
13	7.5	40	0.3	1	4	8.87	8.9	-0.031
14	7.5	20	0.7	1	4	8.43	8.42	0.011
15	7.5	40	0.7	10	4	8.52	8.42	0.103
16	6	40	0.3	1	2	7.72	7.79	-0.073

Table 2 displays the F-test, analysis of variance (ANOVA), and P-value of a selected factor for examination. These tests examine the model's statistical significance. The model is highly significant, as indicated by the model's F value of 17.41 and the low P value of 0.0001. This is evident because the model has a low P value. The model's reliability is determined by calculating the correlation coefficient (R^2: 0.8132, which is near to 1) and the adjusted correlation coefficient (Adj R^2: 0.7665), which indicates that 76.65 percent

of the total variance in response data is accounted for. The sufficiency accuracy result of 11.761 shows that the model has an adequate signal that may be used to traverse the design space. Moreover, the significance of model terms is confirmed by P-values 0.05; in this example, A, C, and E were significant model terms, and there were no significant interacting parameters. The pH p-value is marginally significant, whereas incubation time was the most important variable.

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

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.81	3	1.27	17.41	0.0001	significant
A-pH	0.4131	1	0.4131	5.67	0.0347	
C-Acrylamide	0.9230	1	0.9230	12.66	0.0039	
E-Incubation time	2.47	1	2.47	33.90	< 0.0001	
Residual	0.8749	12	0.0729			
Cor Total	4.68	15				
Std. Dev.	0.2700	R^2	0.8132			
Mean	8.11	Adjusted R^2	0.7665			
C.V. %	3.33	Predicted R^2	0.6679			
		Adeq Precision	11.7612			

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 3. Coded and actual factors for the predicted bacterial growth final equations.

Coded Growth	Factor	Actual Growth	Factor
+8.11		+6.08253	
+0.1607	A	+0.214250	pH
-0.2402	C	-1.20094	Acrylamide
+0.3931	E	+0.393062	Incubation time

The variance inflation factor, or VIF, is a statistic that measures the extent to which a lack of orthogonality in the design raises the variance of a particular model variable. **Table 4** lists the estimated coefficients of the examined components, together with their associated standard errors, confidence intervals, and variance inflation factors (VIF). Only incubation time and pH had positive coefficients among the selected components, with incubation time having a bigger positive value than pH. This indicates that both parameters have a positive effect on the growth of this bacterium on acrylamide, with the incubation time having a greater positive effect or influence than the other variable. In contrast, the coefficient estimate of the acrylamide concentration displays a negative value, indicating that a higher concentration of acrylamide than the optimal is detrimental to the growth of this bacterium when it is fed acrylamide.

The standard error for a model coefficient in an orthogonal design is bigger than the standard error for the same model coefficient in a VIF design by a factor equal to the square root of the VIF. A VIF of 1 is considered desirable since it implies that the coefficient is orthogonal to the other model components, or that the correlation coefficient is 0. In contrast, VIFs with a count larger than 10 may trigger red signals. In addition, VIFs more than one hundred are cause for concern because they show that coefficients were wrongly estimated due to multicollinearity, whilst VIFs greater than one thousand imply severe collinearity. The variance inflation factor (VIF) was found to have a value of 1, indicating that the regression analysis has a substantial amount of multicollinearity [33–35]. Based on the acquired result, only three of the five screened parameters constitute major influential variables, as determined by a two-level factor analysis.

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	8.11	1	0.0675	7.96	8.25	
A-pH	0.1607	1	0.0675	0.0136	0.3078	1.00
C-Acrylamide	-0.2402	1	0.0675	-0.3873	-0.0931	1.00
E-Incubation time	0.3931	1	0.0675	0.2460	0.5401	1.00

It is evident from the Pareto charts developed for the statistical significance analysis of each response coefficient, which is depicted in **Fig. 1**. Bonferroni limit line (t-value of effect: 3.649) and t-limit line is the two limit lines used to classify the t-value of the effect in the Pareto chart (t-value of effect: 2.719). There are three basic categories for determining coefficients' significance. The first coefficient whose t-value of effect is more than the limit established by Bonferroni is considered the most significant, which was the incubation duration factor.

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, of which acrylamide concentration is the factor in this range and pH barely fits in, 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, of which the rest of the factors, which include glucose concentration, could be eliminated. The t-values of both of these coefficients fall between the Bonferroni line and the t-limit line. These results are comparable to those obtained using the coefficient estimate.

The concentration of acrylamide, pH, and period of incubation were the most influential factors in the cellular growth of this bacterium on acrylamide. In a number of OFAT-based methods, these features have been identified as critical for the high growth of microbes on acrylamide. This research was conducted using acrylamide concentrations well within the range known to be tolerable for the vast majority of microorganisms capable of acrylamide breakdown. Acrylamide concentrations above 1000 mg/L are typically toxic to bacteria that degrade acrylamide [16–25,36–42].

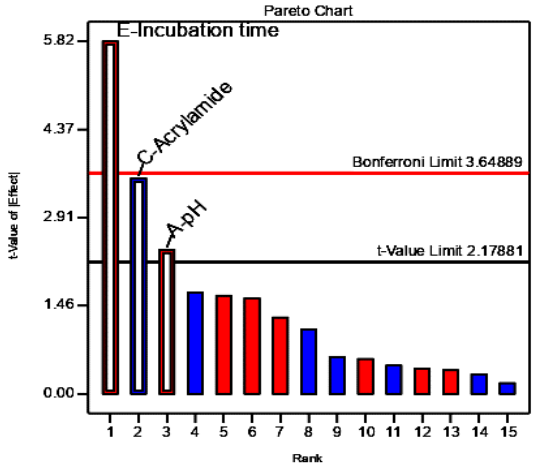

Fig. 1. Pareto chart of operational parameters.

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 absence of interaction between the factors. Interacting effects is a feat that the Plackett-Burman screening method would not be able to detect [43–46].

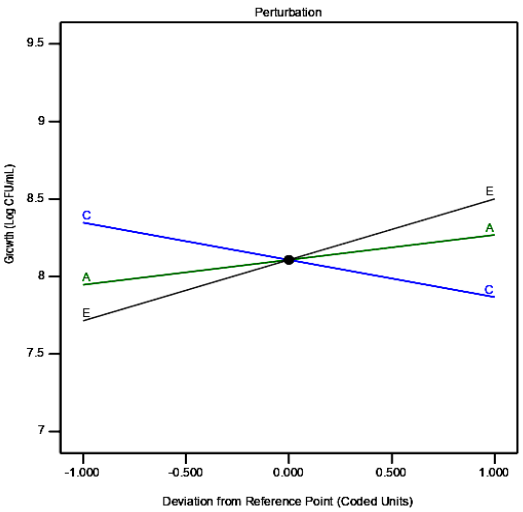

Fig. 2. Perturbation plot of operational parameters obtained through regular two-factor design.

In this regard, a half-normal probability plot of the residuals was constructed and examined (shown in **Fig. 3**) to validate the normality assumption. All internally studentized residuals values were determined to be within 2 and along the straight line, indicating that a transformation of the response is unnecessary. This was discovered through investigation. As depicted in **Fig. 4,** there is a good correlation between the actual experimental results and the values predicted by the model. The Box–Cox figure, depicted in **Fig. 5**, provides useful information for selecting the right power law transformation based on lambda value.

Due to the fact that the 95 percent confidence interval has a value of 1 that corresponds to the value that was designed into the model, it is not advisable that any further alterations be performed to the observed response in order to suit the model. The leverages versus run plot depicted in **Fig. 6** demonstrates that all acquired numerical values fit within the typical range of 0 to 1. This implies that a design point may influence how well the model fits. If there is a problem with the data point, such as an unexpected error, a leverage point value greater than one is regarded "poor" since the error has a considerable effect on the model. According to the plot of leverages vs runs, there are no data with leverages greater than the average leverage, as such data would influence at least one model parameter. A measurement of the response outlier that is equivalent to an experimental trial may be obtained from the plot of Cook's distances (**Fig. 7**).

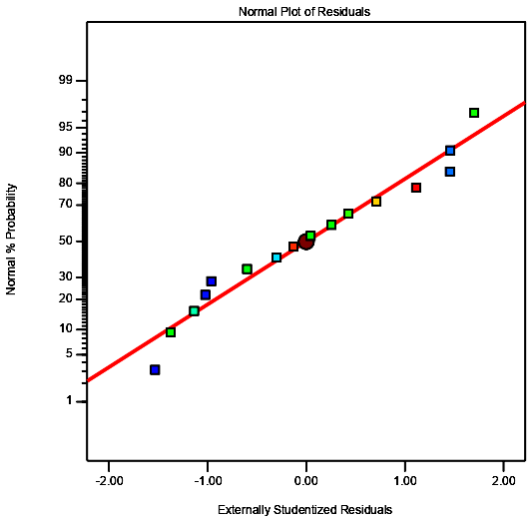

Fig. 3. Diagnostic's plot in the form of the normal plot of residuals for the two level factorial optimization studies.

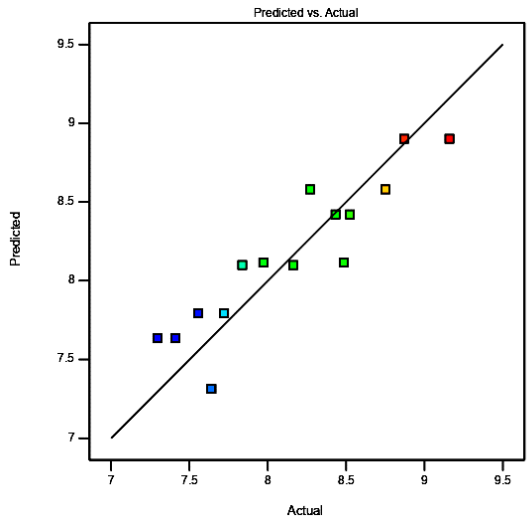

Fig. 4. Diagnostic's plot in the form of the predicted versus actual plot for the two level factorial optimization studies.

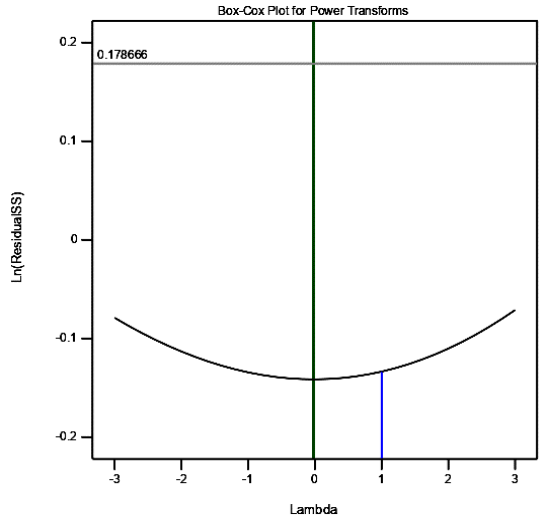

Fig. 5. Diagnostic's plot in the form of Box-Cox plot for the two level factorial optimization studies.

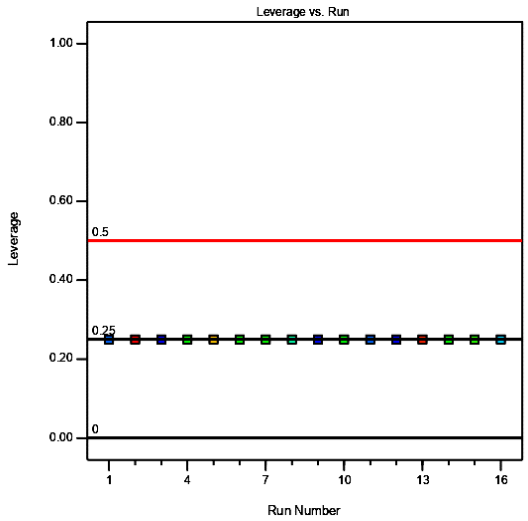

Fig. 6. Diagnostic's plot in the form of leverage vs runs for the two level factorial optimization studies.

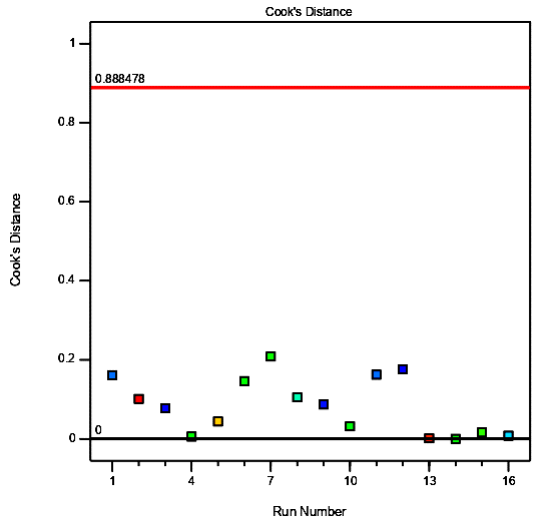

Fig. 7. Diagnostic's plot in the form of Cook's distance vs runs for the two level factorial optimization studies.

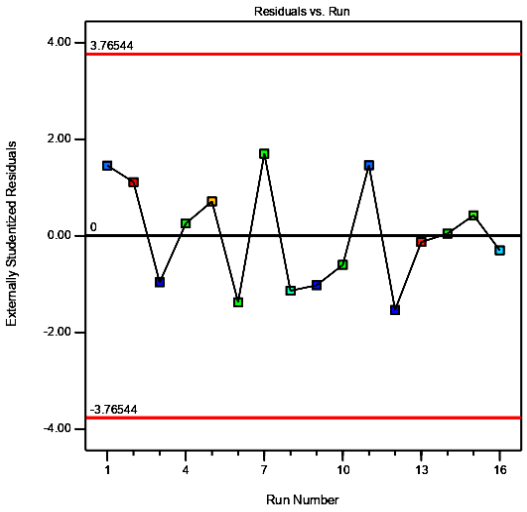

Fig. 8. Diagnostic's plot in the form of residuals vs runs for the two level factorial optimization studies.

Cook's distances are nonnegative numbers, and the greater these numbers, the more meaningful an observation. For the majority of researchers, the threshold for assessing whether or not an observation can be considered relevant is three times the dataset's mean value of Cook's D. The values of Cook's distances are found to be within 1, and the diagnosis does not suggest any transformation strategies. The absence of serial correlation in the residuals vs run data plot (**Fig. 8**) suggests that the characteristics of the data are random [26,27,30,47,48].

It's not always an issue when influential points are raised, but it's crucial to follow up on observations tagged as extremely influential. A high result on an influence measure may suggest a number of factors, such as a data entry error or an observation that is clearly not representative of the population of interest and must therefore be eliminated from the study. During the process of fitting a model, the inclusion of one or more sufficiently critical data items may cause coefficient estimations to be thrown off and the interpretation of the model to become muddled. In the past, before doing a linear regression, histograms and scatterplots were used to assess the likelihood of outliers in a dataset. Before conducting the linear regression, this was completed. Both ways of evaluating data points were subjective, and there was little way to determine the influence of each potential outlier on the data reflecting the results.

This led to the creation of other quantitative indicators, including DFFIT and DFBETA. The DFFFITS algorithm determines how big of an impact each specific example has on the expected value. According to Cook, it is possible to translate it to a distance. In contrast to Cook's distances, DFFITS can be either positive or negative. When the value is zero, the question point is precisely on the regression line. Utilizing leverage makes this possible. It is the mathematical difference between the expected value with observations and the forecast value without observations. DFFITS is the externally studentized residual (ti) multiplied by strong leverage points and reduced by low leverage points, according to the alternative formula [46,49,50]. The plots show the DFBETAS values (**Fig. 9**) were within the size-adjusted threshold acceptable range while the DFFITS values were within the cut-off values (**Fig. 10**).

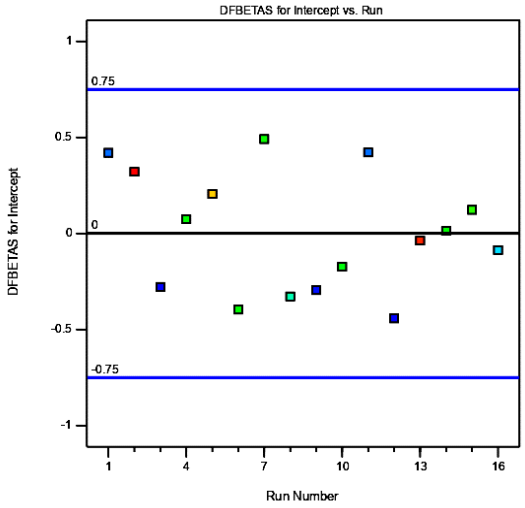

Fig. 9. Diagnostic's plot in the form of DFBETAS for intercept vs runs for the two level factorial optimization studies.

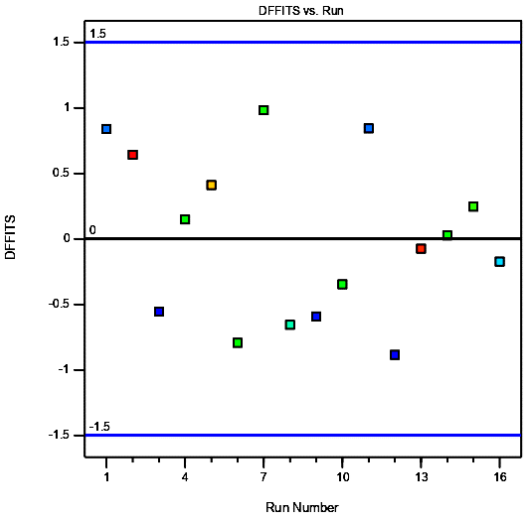

Fig. 10. Diagnostic's plot in the form of DFFITS vs runs for the two level factorial optimization studies.

CONCLUSION

Five independent parameters that influence the development of the bacterium on acrylamide were evaluated using a two-level factorial design. Among these variables are pH, temperature, incubation period, acrylamide content, and glucose concentration. The two-factor factorial design was successful in identifying significant contributing parameters to the cellular growth of this bacterium on acrylamide, namely acrylamide concentration, pH, and incubation duration, which can be adjusted using RSM in future studies. Using ANOVA, Pareto's chart, perturbation's plot, and other diagnostic plots, the significant contributing factors or parameters were examined. Half-normal, Cook's distance, residual vs runs, leverage versus runs, Box-Cox, DFFITS, and DFBETAS diagnostic plots all supported the two-level factorial result. This work was conducted using acrylamide concentrations well within the known tolerance range of most acrylamide-degrading bacteria. Incubation time is an expected consequence, as longer incubation time permits more growth, and incubation times ranging from two to five days for optimal development have been recorded for a variety of acrylamide-degrading bacteria. The majority of acrylamide-degrading microorganisms thrive under near-neutral circumstances, as indicated by the results of our investigation, which are consistent with previous literature trends.

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