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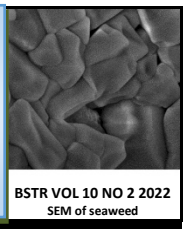

A Two-level Factorial Design for Screening Factors that Influence the Growth of *Staphylococcus* sp. strain Amr-15 on Acrylamide

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ABSTRACT

Due to the fact that it breaks down into acrylamide over time, polyacrylamide is one of the most important sources of acrylamide in soil. As a strategy for bioremediation, the breakdown of acrylamide by the action of microbes has seen a gradual but consistent increase in attention all over the world. A previously isolated molybdenum-reducing bacterium with amide-degrading capability was further identified on significant parameters contributing to optimized growth on acrylamide using a two-level factorial design in this study. The two-level factorial design was adopted in the screening of five independent factors influencing the growth of the bacterium on acrylamide. These factors include pH, temperature, incubation time, acrylamide concentration and glucose concentration. The two-factor factorial design was successful in finding important contributing parameters in the growth of this bacterium on acrylamide, which were acrylamide concentration, pH and incubation time ($p<0.05$) that can be further optimized using RSM in future works. The important contributing factors or parameters were analysed using ANOVA, Pareto's chart and perturbations plot and other diagnostic plots. The diagnostic plots such as half-normal, Cook's distance, residual vs runs, leverage vs runs, Box-Cox, DFFITS, DFBETAS all supported the two-level factorial conclusion. This study was carried out using an acrylamide range well within the range reported to be tolerated by most acrylamide-degrading microorganisms. Incubation time is an expected result since longer incubation time allows more growth and incubation time ranging from two to five days for optimized growth has been reported in many acrylamide-degrading microorganisms. Most of the acrylamide-degrading microorganisms grow well in near-neutral conditions, of which the results obtained in this study conforms to the published literature trends.

INTRODUCTION

Despite the fact that it has been established that exposure to acrylamide in experimental animals might result in the development of cancer [1], evidence in humans who have been exposed to the chemical are starting to become apparent [2]. According to the findings of research that was carried out on the topic, there is a correlation between acrylamide exposure and an increased risk of perinatal death, mutagenicity, clastogenicity, endocrine-related malignancies, and male reproductive toxicity in rats [3]. Because of the histological abnormalities in the seminiferous tubules that are generated by acrylamide, the reproductive systems of male rats are also negatively impacted by this chemical. The chemical is to blame for these anomalies that can be seen in histology. If acrylamide is breathed in or absorbed through the skin, it may give the user a burning sensation or cause a rash to appear. Both of these reactions are conceivable. A tingling tongue, an overactive sweating gland, and a sluggish physique are all indications that something is amiss with the neurological system [1]. According to Yang et al. [4], *Salmonella* strains TA100 and TA98 have been subjected to acrylamide and have been shown to be susceptible to the development of mutations as a result of exposure to the chemical. After the medicine was given, an increased number of

chromosomal abnormalities were observed in the bone marrow of mice that had received an intraperitoneal injection of acrylamide at a dose of 50 mg/kg. The injection was given to the mice in order to study the effects of the drug. The incidence of chromosomal aberrations in lymphocytes obtained from mice that were given intraperitoneal dosages of acrylamide up to 125 mg/kg did not significantly increase when the acrylamide was provided in this manner. This indicates that acrylamide did not enter the lymphocytes through the digestive tract. This result was observed after the acrylamide had been given via the intraperitoneal route [5].

When foods that are high in carbohydrates are cooked at a high temperature, a chemical process that is known as the Maillard reaction may take place. Acrylamide, a molecule that is capable of causing cancer as well as damage to the nervous system, could be formed as a result of this interaction. The Maillard reaction has the potential to produce acrylamide in some foods, particularly those that include a lot of carbohydrates. These are the types of meals. When sugars and amino acids are combined in the correct amounts, a chemical process known as the Maillard reaction will take place. This is the initial step in a series of processes that will ultimately lead to the production of acrylamide [6]. On the other hand, acrylamide may be produced from a variety of other carbonyl compounds [7]. Cattle and fish both perished in Sweden and Norway as a direct result of acrylamide contamination in streams in the surrounding area. In the manufacturing of adhesives, plastics, and printed materials, as well as for the treatment of drinking water, the most common application for acrylicamide is in the formation of polyacrylamide, abbreviated as PAM.

As of the year 2005, commercial polyacrylamides are frequently tainted by the toxic monomer of acrylamide. This situation has had a significant impact on our food supply chain as a direct result of the widespread use of these substances, and it is a direct result of the fact that polyacrylamides are commercially available. A concentration of thirty percent polyacrylamide may be found in the herbicide Roundup, which is responsible for the contamination of agricultural soil with acrylamides. In order to address this issue, which has to be addressed in order to be fixed, the acylamide in the environment needs to be remediated by a biological process [8].

Acrylamide, which has a high solubility in water, may be absorbed via the skin, the lungs, the digestive system, and even the placental barrier. Its ability to dissolve in water gives it this versatility. By analyzing the quantity of acrylamide adducts that are present in haemoglobin, it is feasible to calculate the amount of acrylamide that members of the general public are subjected to as a direct result of the occupations that they have. According to the findings, a total of 41 employees working in an acrylamide manufacturing plant had levels of neurotoxicity that were linked to the biomarker haemoglobin adducts. In the Chinese factory that produces acrylamide, the levels of haemoglobin adducts increased, which is an indication that the employees had been exposed to exceptionally high amounts of acrylamide [9]. Multiple cases of acute acrylamide poisoning have been reported in Japan as a consequence of acrylamide contamination in the country's water supply. Igisu *et al*. [10] reported that a well that had been polluted by a grouting operation that was 2.5 meters deep had an acrylamide content that was as high as 400 mg acrylamide/L. This finding was made after the well had been tested. According to the findings, five people who drank the acrylamide-poisoned drinking water experienced symptoms such as truncal ataxia and disorientation.

Acrylamide enters the body either by breathing in air that is polluted or ingesting or drinking anything that is contaminated in some manner. It is then either be absorbed via the mucous membranes found in the lungs, the digestive system, or the skin. On the other hand, it will be flushed out of the body once it has been metabolized [11–13]. The presence of acrylamide in biological fluids and the dispersion of acrylamide throughout the body both contribute to the facilitation of the impact that acrylamide has. Acrylamide is present in biological fluids. In spite of the fact that it is rapidly metabolized and eliminated after being exposed to it, acrylamide poses a risk to people and employees due to the high degree of reactivity it exhibits toward proteins. This is the case even though it is quickly metabolized and eliminated after being exposed to it. Because of this, researchers have been motivated to develop ways to eliminate acrylamide, particularly the pollution it causes in soils. However, acrylamide remediation in soils is challenging, if not impossible, due to the complex matrix of the soil.

The utilization of microorganisms in the degradation and cleaning up of acrylamide is attractive due to the fact that the metabolism of microorganisms, particularly under aerobic circumstances, permits the complete conversion of acrylamide to non-hazardous water and carbon dioxide. Microorganisms with acrylamide-degrading and assimilating ability are reported in the literature including the yeast *Rhodotorula* sp. [14], the fungi *Aspergillus oryzae* [15] but bacteria remains the most reported degraders of acrylamide [16–25]. Growth experiments on acrylamide require the controlling factors to be optimized.

In fundamental research, the planning of experiments frequently takes an "intuitive" approach. Experiments in biology have always been conducted on a "one factor at a time" basis (OFAT). In this method, all of the factors and variables are kept the same, with the exception of the thing that is being investigated, and that thing's output is analyzed. This strategy has the potential to disclose significant "major effects" in biological research, however, the interactions between components will result in incorrect words. Due to the intricacy of the process, regulating a large number of input factors is required in order to get optimal results. The results of an experiment could be noisy, and there might be a lot of intriguing data coming in. In situations like this, the selection of data points may be tweaked to optimize the amount of relevant information obtained through the use of statistically based experimental design, which can result in significantly more interesting data.

The basic issue structure utilized by the DOE takes into account a number of aspects that are thought to impact process output. The design of the experiment that is ultimately selected is determined by which of several feasible designs yields the most amount of expected information. This criterion is frequently determined according to the precision or accuracy of the fitted model's estimates of the input variable or its forecasts of the output variable. In most cases, the dynamics of this partnership are a mystery. Even though numerous research on process optimization has employed OFAT to increase responsiveness, it will be important to understand the connections between components in order to optimize increasingly complicated procedures. Using an OFAT strategy, one axis would be optimized first, followed by the other. If by some stroke of good fortune, the beginning of the investigation was reasonable in the first place, then the global maximum that maximizes the output variable may be identified. One thing to keep in mind, though, is that there is a significant possibility that the search was terminated at a local maximum or pseudo-optimum.

The Plackett-Burman (PB) experimental design is a prominent screening approach that is used to uncover key components early on in the experimentation phase when comprehensive system knowledge is typically lacking. This method was named after its creators, Plackett and Burman. It was developed in 1946 by statisticians Robin L. Plackett and J.P. Burman with the goal of identifying active variables with the fewest feasible experiments.

Two-factor interactions can be confusing to major effects when using a Plackett-Burman design. When there is little to no potential for two-way interaction, these are the kinds of designs that should be employed. Although the Plackett-Burman design is helpful in detecting large main effects in two-level multi-factor experiments with more than four factors, 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. Because it takes into account how the different components interact with one another, the two-level factorial design is a superior strategy to the PB method in the screening step.

Using this method results in a more accurate estimation of the optimal condition and calculates the interconnections between significant cultural factors. In the literature, numerous screening processes have benefitted from two-level factorial design [26,26–31]. Here we describe the use of a two-level factorial design to screen for significant factors that influence the growth of *Staphylococcus* sp. strain Amr-15 on acrylamide.

MATERIALS AND METHODS

In the course of the investigation, all of the chemical reagents were utilized in their unpurified states even though they had been manufactured in substantial quantities. In addition, the analytical quality of all of the materials that were used in this inquiry was preserved throughout the entirety of the process. Experiments were carried out in triplicate in each and every instance unless otherwise noted in the notes that accompanied the study.

Growth and maintenance of acrylamide-degrading bacterium

The bacterium was previously isolated from soil samples obtained from the grounds of a polluted site in Sadat City, Egypt. The isolation, partial identification and characterization of the growth based on five operational parameters (pH, temperature, incubation time, acrylamide concentration and glucose concentration) of this bacterium on acrylamide are reported elsewhere. Growth and maintenance of the bacterium were conducted on minimal salts medium (MSM) supplemented with only acrylamide as the source of nitrogen and glucose as the sole carbon source.

Revival of the bacterium from a 16% glycerol stock was carried out by growing overnight the pure culture in 10 mL of nutrient broth. From this, 0.1 mL was added into 45 mL of acrylamide enrichment medium in a 100 mL volumetric flask and the culture was incubated at 150 rpm for 48 h at 25 ℃ on an incubator shaker (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 acts as a buffer system, maintaining a pH range that spans from 5.8 to 7.8. Acrylamide was the only source of nitrogen that was employed for the sterilisation process, and PTFE syringe filters with a pore size of 0.45 microns were used. In order to determine the number of bacteria present, samples of one millilitre each were successively diluted in sterile tap water and plated on nutrient agar. The presence of phosphate in the medium acts as a buffer system, maintaining a pH range that spans from 5.8 to 7.8. Acrylamide was filter-sterilized using PTFE syringe filters with a pore size of 0.45 microns. In order to determine the number of bacteria present, samples of one millilitre each were successively diluted. In order to determine the number of bacteria present, samples of one millilitre each were successively diluted in sterile tap water and plated on nutrient agar overnight.

Screening of significant parameters using two-level factorial design

The two-level factorial design was utilized for the primary purpose of determining the relative importance of several different factors that had an effect, despite the presence of intricate interactions. We carried out the 2-factorial design with a total of five components. The lower value was represented by code -1, and the greater value was represented by code 1. The response was bacterial growth, which was determined using the log CFU/mL scale.

The tests were planned and carried out in accordance with the sequence that is presented in **Table 1**. Every experiment was carried out in triplicate, and the results of both sets are shown below along with their means. In order to determine which of these parameters is significantly more important than the others, the data were run through software (Design Expert 7.0, Stat-Ease, Inc.'s (trial version).

Table 1. Coded and actual values of the experiment.

Factor	Name	Units	Mini-mum	Max-imum	Coded Low	Coded High	Mean	Std. Dev.
A	pH		6.50	7.50	-1 ↔ 6.50	+1 ↔ 7.50	7.00	0.516
B	Temperature	ºC	25.0	40.00	-1 ↔ 25.0	+1 ↔ 40.0	32.50	7.75
C	Acrylamide	g/L	0.30	1.00	-1 ↔ 0.30	+1 ↔ 1.00	0.65	0.362
D	Incubation time	Days	2.00	4.00	-1 ↔ 2.00	+1 ↔ 4.00	3.00	1.03
E	Glucose	g/L	5.00	10.00	-1 ↔ 5.00	+1 ↔ 10.00	7.50	2.58

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 significant. Whenever appropriate, values will be truncated to three decimal points.

RESULTS

Two-level factorial design for screening the operational factors

In the 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 7.66 log CFU/mL to 9.55 log CFU/mL. **Table 2** 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 residual.

Table 2. Two-level factorial design for screening best parameters for the growth of *Staphylococcus* sp. strain Amr-15.

Run	A: pH	B: Tempe-rature	C: Acry-lamide	D: Incuba-tion time	E: Glucose	Growth (Log CFU/mL)	Predicted growth (Log CFU/mL)	Resi-dual
1	6.5	25	1	2	5	8.07	7.88	0.19
2	7.5	25	0.3	4	10	9.55	9.14	0.41
3	7.5	25	1	2	10	7.83	7.88	-0.05
4	6.5	25	1	4	10	8.55	8.66	-0.11
5	6.5	40	0.3	4	10	9.18	9.14	0.04
6	6.5	25	0.3	4	5	8.66	9.14	-0.48
7	7.5	25	0.3	2	5	8.9	8.35	0.55
8	6.5	40	1	4	5	8.23	8.66	-0.44
9	6.5	25	0.3	2	10	7.97	8.35	-0.39
10	7.5	40	0.3	2	10	8.34	8.35	-0.02
11	6.5	40	1	2	10	8.06	7.88	0.19
12	7.5	40	1	2	5	7.66	7.88	-0.21
13	7.5	40	0.3	4	5	9.29	9.14	0.15
14	7.5	25	1	4	5	8.79	8.66	0.13
15	7.5	40	1	4	10	8.96	8.66	0.30
16	6.5	40	0.3	2	5	8.08	8.35	-0.27

The F-test, analysis of variance (ANOVA), and the P-value of a chosen factor are shown in **Table 3** for evaluation. These tests evaluate the statistical significance of the model. The findings showed that the model is highly significant, as shown by the F value of 15.96 and the low P value of 0.0003. This is clear from the fact that the model has a low P value. Calculating the correlation coefficient (R2: 0.7107, which is closer to unity) and the adjusted correlation coefficient (Adj R2: 0.6661), which implies that 66.61 percent of the overall variance in response data, are used to verify the model's dependability. The result for the "**Adeq Precision"** was found to be 8.9602, which indicates that the model has an appropriate signal that can be utilized to traverse the design space. Moreover, the significance of model terms is verified by P-values <0.05 and in this case, C-Acrylamide and D-Incubation time were significant model terms and the absence of interacting significant parameters. Incubation time was the most significant factor.

Table 3. Analysis of variance (ANOVA) for regular two-level factorial analysis.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.38	2	1.69	15.96	0.0003	significant
C-Acrylamide	0.9073	1	0.9073	8.56	0.0118	
D-Incubation time	2.48	1	2.48	23.37	0.0003	
Residual	1.38	13	0.1060			
Cor Total	4.76	15				
Std. Dev.	0.3256	R^2	0.7107			
Mean	8.51	Adjusted R^2	0.6661			
C.V. %	3.83	Predicted R^2	0.5617			
		Adeq Precision	8.9602			

By applying a 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 4**).

Table 4. Coded and actual factors for the predicted bacterial growth final equations.

Coded		Actual	
Growth	Factor	Growth	Factor
+8.507375		+7.76911	
-0.23813	C	-0.68036	Acrylamide
+0.3935	D	+0.3935	Incubation time

Table 5 contains an inventory of the estimated coefficients of the components that were investigated, as well as their associated standard errors, confidence limits, and variance inflation factors (VIF). In the group of selected components, only incubation time and pH exhibit positive coefficients, with incubation time providing a greater positive value than pH. This suggests that both parameters have a beneficial effect on the development of this bacteria on acrylamide, with the incubation duration having a higher beneficial effect or influence than the other element. On the other hand, the coefficient estimate of the acrylamide concentration reveals a negative value, which suggests that a greater acrylamide concentration than the ideal is detrimental to the growth of this bacteria when it is fed acrylamide.

The variance inflation factor, or VIF, is a statistic that determines how much a lack of orthogonality in the design increases the variance of a certain model coefficient. When specifically comparing the standard error for a model coefficient in an orthogonal design to the standard error for the same model coefficient in a VIF design, the standard error for the VIF design is greater by a factor equal to the square root of the VIF. In general, a VIF of 1 is considered to be optimal since it indicates that the coefficient is orthogonal to the other model components; in other words, the correlation coefficient is 0. On the other hand, VIFs that are greater than 10 might raise some red flags.

In addition, VIFs that are greater than one hundred are the reason for concern since they indicate that coefficients were calculated incorrectly owing to multicollinearity, and VIFs that are greater than one thousand are the result of severe collinearity. The value of the variance inflation factor (VIF) was found to be 1, which suggests that the regression analysis has a significant amount of multicollinearity [32–34]. Based on the result obtained, out of five screened parameters, only three form a major influential factor as obtained through two-level factor analysis.

Table 5. Coefficient estimate obtained during ANOVA for two-level factorial design.

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	8.51	1	0.0814	8.33	8.68	
C-Acrylamide	-0.2381	1	0.0814	-0.4140	-0.0623	1.00
D-Incubation time	0.3935	1	0.0814	0.2177	0.5693	1.00

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: 3.584) 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: 2.610). 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 most significant, which was the factor incubation period. 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 fall into. Both of these coefficients have t-values that are between the Bonferroni line and the t-limit line. These findings are similar to what was found when using the coefficient estimate.

The acrylamide concentration and incubation duration were the main contributing parameters in the development of this bacteria 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 acrylamide-degrading microorganisms [16–25,35–41].

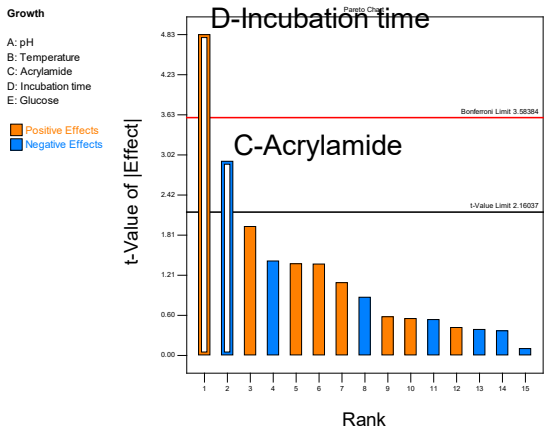

Fig. 1. Pareto chart of operational parameters.

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 D (incubation time) has a similar steep slope to factor C (acrylamide) in an opposing manner. 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 [42–45].

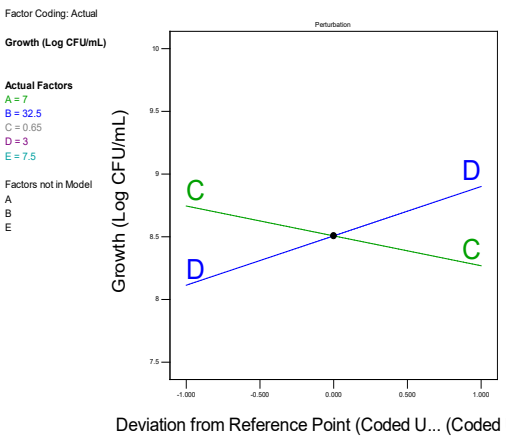

Fig. 2. Perturbation plot of operational parameters obtained through regular two-factor design.

In this regard, a half-normal probability plot of the residuals was generated and evaluated (shown in **Fig. 3**) in order to verify that the normality assumption was accurate. All of the internally studentized residuals values were found to be within 2 and along a straight line, which suggests that there is no requirement for a transformation of the response. This was discovered through research. As can be seen in **Fig. 4**, the graph comparing the actual experimental results to the values predicted by the model indicates that there is a strong match. The Box–Cox plot, which can be found in **Fig. 5**, offers helpful guidance for choosing the appropriate power law transformation based on the value of lambda. Due to the fact that the 95% confidence interval has a value of 1 that corresponds to the value that was designed into the model, it is not advised that any further transformations be made to the observed response in order to fit the model. The leverages vs run plot shown in **Fig. 6** reveals that all of the acquired numerical values fall within the usual limits range of 0–1. This indicates the possibility that a design point will have an effect on how the model fits. If there is an issue with the data point, such as an unanticipated error, a high leverage point value of more than one is considered "bad" since the error has a significant impact on the model.

According to the plot of leverages vs runs, there are no data that are higher than the average leverage since data that are higher than this would impact 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**). Cook's distances are values that cannot be negative, and the higher these values are, the more significant an observation is. For the majority of researchers, the threshold for determining whether or not an observation can be considered important is three times the dataset's mean value of Cook's D. The values of the Cook's distances are determined to be within a value of 1, and the diagnosis does not recommend any transformation methods. 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 [26,27,30,46,47].

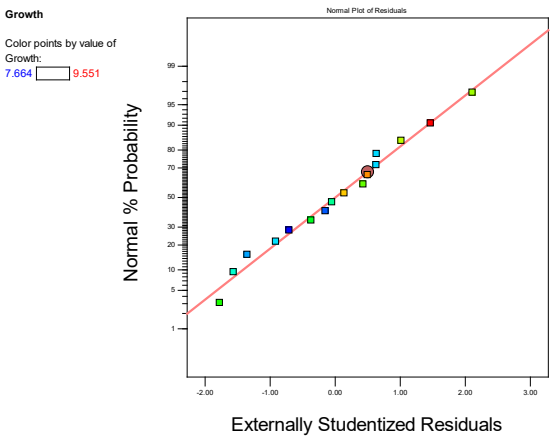

Fig. 3. Diagnostic plot in the form of the normal plot of residuals for the two-level factorial optimization studies.

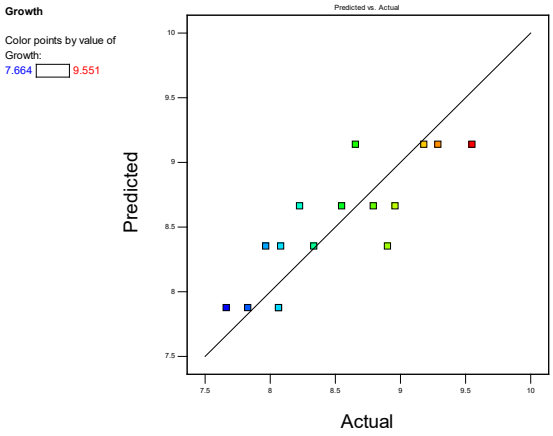

Fig. 4. Diagnostic plot in the form of the predicted versus actual plot for the two-level factorial optimization studies.

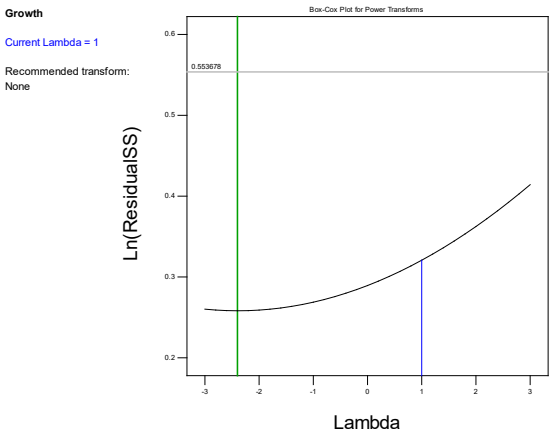

Fig. 5. Diagnostic plot in the form of Box-Cox plot for the two-level factorial optimization studies.

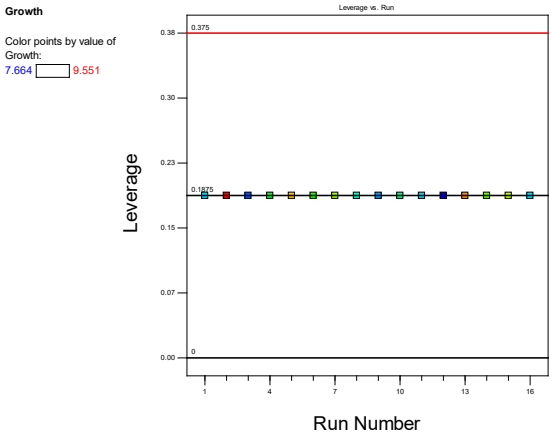

Fig. 6. Diagnostic plot in the form of leverage vs runs for the two-level factorial optimization studies.

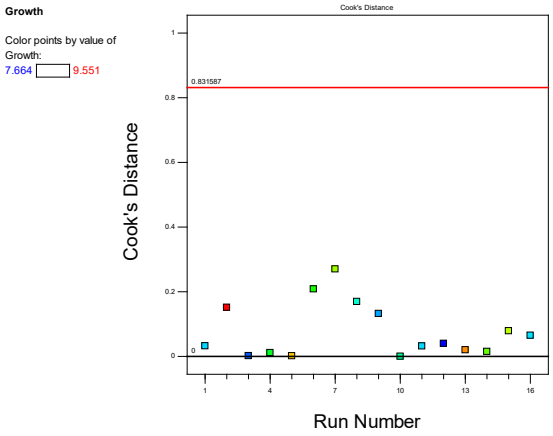

Fig. 7. Diagnostic plot in the form of Cook's distance vs runs for the two-level factorial optimization studies.

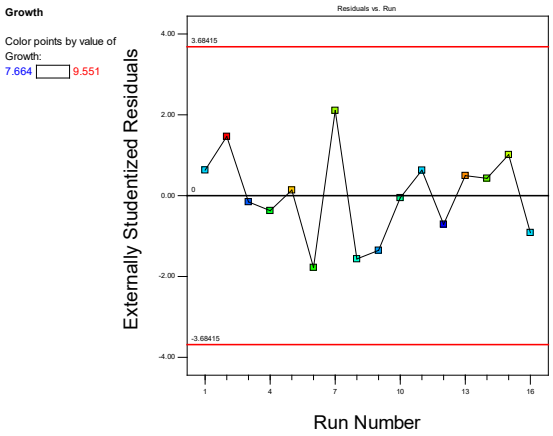

Fig. 8. Diagnostic plot in the form of residuals vs runs for the two-level factorial optimization studies.

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 to 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. 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 [45,48,49]. 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**).

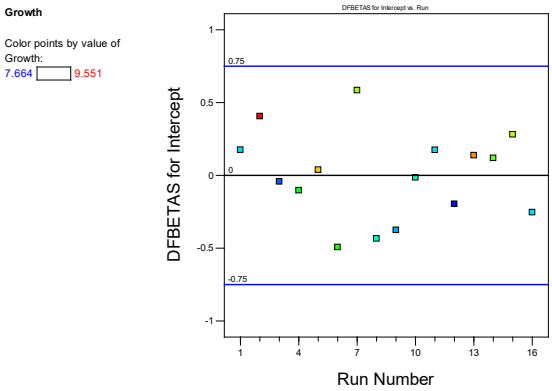

Fig. 9. Diagnostic plot in the form of DFBETAS for intercept vs runs for the two-level factorial optimization studies.

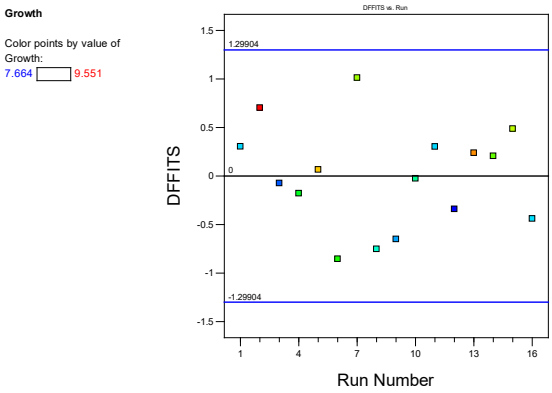

Fig. 10. Diagnostic plot in the form of DFFITS vs runs for the two-level factorial optimization studies.

CONCLUSION

The two-level factorial design was adopted in the screening of five independent factors influencing the growth of the bacterium on acrylamide. These factors include pH, temperature, incubation time, acrylamide concentration and glucose concentration. The two-factor factorial design was successful in finding important contributing parameters in the growth of this bacterium on acrylamide, which were acrylamide concentration, pH and incubation time that can be further optimized using RSM in future works. The important contributing factors or parameters were analysed using ANOVA, Pareto's chart and perturbations plot and other diagnostic plots. The 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 conclusion. This study was carried out using an acrylamide range well within the range reported to be tolerated by most acrylamide-degrading microorganisms. Incubation time is an expected result since longer incubation time allows more growth and incubation time ranging from two to five days for optimized growth has been reported in many acrylamide-degrading microorganisms. Most of the acrylamide-degrading microorganisms grow well in near-neutral conditions, of which the results obtained in this study conforms to published literature trends.

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