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A Two-Level Factorial Design for Screening Factors that Influence the Growth of *E. cloacae* strain UPM2021a on Acrylamide

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ABSTRACT

The world has gradually but steadily paid more attention to the use of bacteria to break down acrylamide as a bioremediation technique. Using a two-level factorial design, a previously obtained molybdenum-reducing bacterium with the ability to degrade amides was further identified on important factors influencing its optimal development on acrylamide. In order to screen five distinct parameters impacting the development of the bacterium on acrylamide, a two-level factorial design was used. Three center point replications were used in a total of 32 tests. These variables include pH, temperature, the length of the incubation period, the concentrations of acrylamide and glucose. Acrylamide concentration, pH, and incubation time were found to be key factors in this bacterium's growth on acrylamide by the two-factor factorial design and were successfully adjusted using RSM in subsequent studies. Using ANOVA, Pareto's chart, pertubation's plot, and other diagnostic plots, the significant contributing components or parameters were analyzed. The two-level factorial conclusion was supported by diagnostic plots including half-normal, Cook's distance, residual vs runs, leverage vs runs, Box-Cox, DFFITS, and DFBETAS. The acrylamide range used in this investigation is well within the range that most acrylamide-degrading bacteria have been found to tolerate. Longer incubation times allow for higher growth, and many acrylamide-degrading microorganisms have been observed to have incubation times of two to five days for optimized growth. The majority of acrylamide-degrading microorganisms thrive at circumstances that are close to neutral, and the findings of this study are consistent with published literature trends in this regard.

INTRODUCTION

Although it has been proven that exposure to the chemical acrylamide in lab animals may lead to the development of cancer [1], evidence of cancer in humans who have been exposed to the substance is just now starting to emerge [2]. There is a link between acrylamide exposure and a higher risk of perinatal death, mutagenicity, clastogenicity, endocrine-related cancers, and male reproductive toxicity in rats, according to the results of the relevant research [3]. The reproductive systems of male rats are also adversely affected by acrylamide because of the histological abnormalities in the seminiferous tubules that are produced by this chemical. These abnormalities, which are visible on histology, are caused by the chemical. A burning feeling or rash may be experienced by the user if acrylamide is breathed in or absorbed via the skin. These two responses are both plausible. One sign that anything is wrong with the nervous system is a tingling tongue, an overactive sweat gland, and a sluggish body [1]. Salmonella strains TA100 and TA98 that have been exposed to acrylamide have been found by Yang et al. to be prone to mutation formation as a result of the chemical exposure. A greater number of chromosomal abnormalities were found in the bone marrow of mice that had received an intraperitoneal injection of acrylamide at a dose of 50 mg/kg after the medication was administered. In order to research the drug's effects, the mice received the injection. When the acrylamide was administered in

this way, the incidence of chromosomal abnormalities in lymphocytes taken from mice that received intraperitoneal dosages of acrylamide up to 125 mg/kg did not significantly increase. This suggests that the lymphocytes were not exposed to the acrylamide through the digestive system. This outcome was seen following intraperitoneal administration of the acrylamide [5].

A chemical reaction known as the Maillard reaction might occur when meals rich in carbohydrates are cooked at a high temperature. This interaction could result in the formation of acrylamide, a chemical that can harm the nervous system and cause cancer. Acrylamide may be produced by the Maillard process in some meals, especially those high in carbs. These are the different meal types. The Maillard reaction, a chemical reaction, occurs when sugars and amino acids are mixed in the proper ratios. This is the first procedure in a chain of events that will eventually produce acrylamide [6]. However, a number of different carbonyl compounds can be used to make acrylamide [7]. As a direct result of acrylamide contamination in nearby streams, both fish and cattle died in Sweden and Norway.

The most widespread use of acrylamide is in the production of adhesives, plastics, printed materials, as well as for the purification of drinking water. This compound is known as polyacrylamide, or PAM for short. Commercial polyacrylamides are still commonly contaminated with acrylamide's hazardous monomer as of 2005. Because of the extensive usage of these compounds and the availability of polyacrylamides in the market, this situation has had a tremendous influence on our food supply chain. In order to solve this problem, which needs to be addressed in order to be rectified, the acrylamide in the environment needs to be remediated by a biological process [8]. This problem is caused by the herbicide Roundup, which may include a concentration of 30% polyacrylamide [9].

Acrylamide can be absorbed via the skin, lungs, digestive system, and even the placental barrier due to its high solubility in water. Its adaptability is due to its capacity to dissolve in water. It is possible to determine how much acrylamide the general public is exposed to as a direct result of their jobs by looking at the amount of acrylamide adducts that are present in haemoglobin. The research revealed that levels of neurotoxicity in 41 workers at an acrylamide production facility were connected to the biomarker haemoglobin adducts. Haemoglobin adduct levels in the acrylamide-producing Chinese factory increased, which is a sign that the workers there had been exposed to extraordinarily high quantities of acrylamide [9]. As a result of acrylamide contamination in the nation's water supply, numerous cases of acute acrylamide poisoning have been documented in Japan. According to Igisu et al. [10], a 2.5-meter-deep well that had been contaminated by a grouting operation had an acrylamide concentration as high as 400 mg/L. The well was examined after which this discovery was made. Five people who drank the acrylamide-tainted water, it was discovered, showed signs like truncal ataxia and confusion.

Acrylamide enters the body either either ingesting or drinking anything that is tainted in some way or by breathing in contaminated air. Then, it is either absorbed through the skin, the digestive system, or the mucous membranes of the lungs. On the other hand, after it has been digested, it will be eliminated from the body [11–13]. The facilitation of the effect of acrylamide is caused by both the presence of acrylamide in biological fluids and the dispersion of acrylamide throughout the body. Biologic fluids contain acrylamide. Despite being quickly digested and removed after exposure, acrylamide nevertheless poses a risk to people and workers due to the high level of protein reactivity it displays. Despite the fact that it is swiftly digested and removed after exposure, this is the case. As a result, scientists have been inspired to create strategies to get rid of acrylamide, especially the pollution it creates in soils. However, because of the intricate soil matrix, acrylamide remediation in soils is difficult, if not impossible. The use of microorganisms in the breakdown and cleanup of acrylamide is appealing because their metabolism, particularly when grown in aerobic conditions, allows for the entire breakdown of acrylamide into non-hazardous water and carbon dioxide. In the literature, acrylamide-degrading and -assimilating microorganisms have been identified, including the yeast *Rhodotorula* sp. [14] and the fungus *Aspergillus oryzae* [15]. However, bacteria have been identified as the most common acrylamide degraders [16–25]. Optimizing the controlling variables is necessary for acrylamide growth experiments.

The design of experiments in fundamental research typically follows a "intuitive" process. Biology experiments have always been carried out "one factor at a time" (OFAT). With the exception of the object under investigation, all other variables and components are maintained constant in this approach, and the object's output is examined. The interplay between the components will lead to inaccurate language, yet this technique has the potential to reveal important "major impacts" in biological research. Due to the complexity of the process, it is necessary to control a significant number of input elements in order to achieve the best outcomes. There may be a lot of interesting data being collected while the findings of an experiment may be noisy. In circumstances like this, it may be possible to adjust the data point selection to maximize the quantity of pertinent information gathered by the use of statistically based experimental design, which can produce noticeably more interesting data.

Numerous factors that are believed to have an impact on process output are taken into account by the DOE's fundamental issue structure. Which of numerous viable designs produces the greatest quantity of expected information will ultimately be chosen as the experiment's design. The fitted model's estimates of the input variable or its forecasts of the output variable are usually used to determine this criterion. The workings of this collaboration are typically a mystery. Although OFAT has been used in various process optimization studies to improve responsiveness, understanding the relationships between components will be crucial for optimizing ever-more complex processes. An OFAT strategy would optimize one axis before moving on to the other. The global maximum that maximizes the output variable may be found if, by some stroke of luck, the investigation's initial starting point was appropriate. However, it should be noted that there is a good chance that the search was stopped at a local maximum or pseudo-optimum.

Early in the experimentation phase, when thorough system information is often missing, the Plackett-Burman (PB) experimental design is a popular screening method used to identify important components. This approach was given the names Plackett and Burman by its developers. The statisticians Robin L. Plackett and J.P. Burman created it in 1946 with the intention of discovering active variables with the fewest number of practical experiments. Using a Plackett-Burman design, two-factor interactions can be confused to big consequences. These are the kinds of designs that need to be used when there is little to no chance for two-way interaction. The Plackett-Burman design is useful in identifying substantial main effects in two-level multi-factor studies with more than four variables, but because it is the smallest design, not enough data have been

gathered to determine whether one factor's effect depends on another. The two-level factorial design outperforms the PB technique in the screening stage because it considers how various components interact with one another. This approach calculates the relationships between important cultural elements and yields a more precise evaluation of the ideal condition. Numerous screening procedures have benefited from two level factorial design, according to the literature [26,26–31]. Here we describe the use of a two-level factorial design to screen for significant factors that influence the growth of *E. cloacae* strain UPM2021a on acrylamide.

MATERIALS AND METHODS

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 isolated from a paddy field in Kepala Batas, which is located in the state of Penang, Malaysia in 2021. The bacterium was grown on Minimal Salts Medium agar that had been supplemented with 1 percent glucose (w/v) as the carbon source and 0.5 g/L (w/v) of acrylamide as the sole nitrogen source. The culture was then shaken at 150 revolutions per minute (rpm) for 72 hours at a temperature of 25 degrees Celsius (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 functions as a buffer system, keeping the pH within the range of 5.8 to 7.8 all the time. During the sterilization process, the only source of nitrogen that was used was acrylamide, and the PTFE syringe filters that were utilized had a pore size of 0.45 micron. Samples of one milliliter each were successively diluted in sterile tap water and then plated on nutrient agar in order to assess the number of bacteria that were present.

Screening of significant parameters using two level factorial design

The two-level factorial design was implemented for the primary aim of identifying the relative importance of a number of various parameters including pH, temperature, acrylamide, and glucose concentrations. This was accomplished by comparing the results of the design with one another. In this experiment, we used a 2-factorial design with the five components listed above. The value that was lower was represented by the code -1, while the value that was higher was represented by the code 1. The scale for determining log CFU/mL was used to analyze the response, which was the growth of bacteria. The planning and execution of the tests were done in such a way as to adhere to the sequence that is outlined in **Table 1**.

The experiment had a total of 32 different trials, each with three different replications of the center point, and it had two different coded levels that were arranged in a randomized pattern. Every experiment was performed twice, and both sets of results, along with their respective means, are shown in the following table. In order to determine which of these parameters are significantly more important than the others, the data were run through a software (Design Expert 7.0, Stat-Ease, Inc.'s (trial version).

Table 1. Parameters and range studied.

Factor	Name	Units	Mini-mum	Maxi-mum	Coded Low	Coded High	Mean	Std. Dev.
A	pH		6.50	7.50	-1 ↔ 6.50	+1 ↔ 7.50	7.00	0.4851
B	Temperature	°C	25.00	35.0	-1 ↔ 25.00	+1 ↔ 35.00	30.00	4.85
C	Acrylamide	g/L	0.3000	1.0	-1 ↔ 0.30	+1 ↔ 1.00	0.624	0.35
D	Glucose	g/L	3.00	10.0	-1 ↔ 3.00	+1 ↔ 10.00	6.50	3.40
E	Incubation time	Days	1.0000	4.00	-1 ↔ 1.00	+1 ↔ 4.00	2.50	1.46

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

For the purpose of the factor screening study, a regular two-level factorial design was utilized, and the following five operational parameters were taken into consideration: pH, temperature, incubation duration, acrylamide concentration, and glucose concentration. The bacterial growth rate was found to range from 7.21 log CFU/mL all the way up to 9.64 log CFU/mL, which was within the range of minimum and maximum values that were tested. **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 residuals. **Table 2** also includes the values that were obtained from the experiment.

Table 3 provides an evaluation tool that displays the F-test, analysis of variance (ANOVA), and P-value of a selected factor. These tests determine whether or not the model has any significant statistical underpinnings. The findings demonstrated that the model is very significant, as indicated by the high F value of 10.67 and the low P value of 0.0001 in the analysis. The fact that the model has a low P value makes this abundantly evident. Calculating the correlation coefficient (R^2: 0.8163, which is closer to unity) and the adjusted correlation coefficient (Adj R^2: 0.7398), which indicates that 73.98 percent of the overall variance in response data, are used to verify the model's dependability. Both of these coefficients are used in the calculation of the adjusted R^2 value. The value of 13.103 that was discovered for the adequacy accuracy indicates that the model possesses a suitable signal that can be used to navigate the design space. This was discovered after the result for the accuracy of the adequacy was calculated.

Table 2. Two-level factorial design for screening best parameters for the growth of *E. cloacae* strain UPM2021a.

Run	A: pH	B: Temperature	C: Acrylamide	D: Glucose	E: Incubation time	Growth (Log CFU/mL)		
		ºC	g/L	g/L	Days	Log CFU/mL	Predicted growth (Log CFU/mL)	Residual
1	7.5	25	1	10	4	8.561	8.715	-0.154
2	7.5	35	1	10	4	9.39	9.531	-0.141
3	7.5	25	0.3	3	1	8.116	8.385	-0.269
4	7.5	35	0.3	3	1	8.699	8.589	0.110
5	7.5	25	0.3	3	4	8.598	8.731	-0.133
6	7.5	35	1	3	1	8.796	8.733	0.063
7	6.5	25	0.3	10	1	7.636	7.555	0.081
8	6.5	25	1	10	1	8.012	8.377	-0.365
9	7.5	35	0.3	3	4	8.52	8.827	-0.307
10	7.5	35	0.3	10	1	8.677	8.487	0.190
11	6.5	35	1	3	1	8.464	8.482	-0.018
12	6.5	25	1	3	1	8.358	8.278	0.080
13	6.5	25	0.3	10	4	7.456	7.642	-0.186
14	7.5	25	1	10	1	8.81	8.628	0.182
15	7.5	35	1	3	4	9.051	8.971	0.080
16	6.5	35	1	10	4	9.644	9.280	0.364
17	6.5	35	0.3	3	1	7.408	7.660	-0.252
18	7.5	35	0.3	10	4	9.194	9.387	-0.193
19	6.5	35	0.3	10	1	7.207	7.558	-0.351
20	7.5	25	1	3	4	9.218	8.875	0.343
21	7.5	35	1	10	1	8.519	8.631	-0.112
22	7.5	25	0.3	10	4	8.807	8.571	0.236
23	7	30	0.35	6.5	2.5	8.205	8.253	-0.048
24	7.5	25	0.3	10	1	8.49	8.484	0.006
25	6.5	35	1	10	1	8.555	8.380	0.175
26	7	30	0.35	6.5	2.5	8.858	8.253	0.605
27	6.5	25	0.3	3	1	7.835	7.456	0.379
28	6.5	35	0.3	10	4	8.331	8.458	-0.127
29	6.5	25	1	3	4	8.225	8.624	-0.399
30	6.5	25	0.3	3	4	7.894	7.802	0.092
31	7.5	25	1	3	1	8.242	8.529	-0.287
32	6.5	35	1	3	4	8.848	8.720	0.128
33	7	30	0.35	6.5	2.5	8.471	8.253	0.218
34	6.5	25	1	10	4	8.472	8.464	0.008
35	6.5	35	0.3	3	4	7.901	7.898	0.003

In addition, the significance of model terms is determined by P-values that are less than 0.05, and in this particular instance, A-pH, B-Temperature, C-Acrylamide, D-Glucose, and E-Incubation time were significant model terms, in addition to the interacting factors AC, BD, BE, DE, and BDE. However, there were no significant interactions between significant parameters. The p value for pH is just marginally significant, but the length of incubation was the component that made the largest difference.

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

	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8.52	10	0.8518	10.67	< 0.0001	Signi-ficant
A-pH	2.79	1	2.79	34.89	< 0.0001	
B-Temperature	0.6255	1	0.6255	7.83	0.0100	
C-Acrylamide	1.99	1	1.99	24.86	< 0.0001	
D-Glucose	0.0788	1	0.0788	0.9869	0.3304	
E-Incubation time	1.23	1	1.23	15.46	0.0006	
AC	0.9194	1	0.9194	11.51	0.0024	
BD	0.1342	1	0.1342	1.68	0.2072	
BE	0.2489	1	0.2489	3.12	0.0902	
DE	0.0812	1	0.0812	1.02	0.3233	
BDE	0.4241	1	0.4241	5.31	0.0301	
Residual	1.92	24	0.0798			
Lack of Fit	1.70	22	0.0773	0.7170	0.7310	not signif-icant
Pure Error	0.2156	2	0.1078			
Cor Total	10.43	34				

Std. Dev.	0.2826	R^2	0.8163
Mean	8.44	Adjusted R^2	0.7398
C.V. %	3.35	Predicted R^2	0.6265
		Adeq Precision	13.1003

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 4**).

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

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

	=	Actual Growth Equation	=
+8.46		-2.16401	
+0.2951	A	+1.21970	pH
+0.1398	B	+0.045785	Temperature
+0.2416	C	+7.47024	Acrylamide
+0.0496	D	+0.208119	Glucose
+0.1964	E	+0.571054	Incubation time
-0.1695	AC	-0.968571	pH * Acrylamide
+0.0648	BD	-0.007264	Temperature * Glucose
+0.0882	BE	-0.016749	Temperature * Incubation time
+0.0504	DE	-0.121976	Glucose * Incubation time
+0.1151	BDE	+0.004386	Temperature * Glucose * Incubation time

Table 5 includes a listing of the estimated coefficients of the components that were researched, as well as the standard errors, confidence limits, and variance inflation factors that are connected with those estimated coefficients (VIF). Only incubation time and pH display positive coefficients within the group of selected components, with incubation time producing a bigger positive value than pH. The only other component to exhibit a positive coefficient is pH. This would imply that both of these parameters have a good effect on the cellular growth of this bacterium on acrylamide, with the incubation length having a stronger beneficial effect or influence than the other component. On the other hand, the coefficient estimate of the acrylamide concentration reveals a negative value, which indicates that a higher acrylamide concentration than the ideal is detrimental to the growth of this bacterium when it is fed acrylamide. This conclusion can be drawn from the fact that the coefficient estimate of the acrylamide concentration reveals a negative value.

The variance inflation factor, often known as VIF, is a statistic that determines the extent to which the variance of a particular model coefficient is increased due to a lack of orthogonality in the design. When the standard error for a model coefficient in an orthogonal design is specifically compared 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 other words, the standard error for the VIF design is larger. In most cases, a VIF of 1 is thought to be the best possible value because this value implies that the coefficient is orthogonal to the other components of the model; to put this another way, the correlation coefficient is equal to 0. VIFs that are more than 10 may, on the other hand, trigger some warning signals. VIFs that are greater than one hundred are cause for concern because they indicate that coefficients were calculated incorrectly due to multicollinearity, and VIFs that are greater than one thousand are the result of severe collinearity.

In addition, VIFs that are less than one hundred indicate that coefficients were correctly calculated due to multicollinearity. Due to the fact that the value of the variance inflation factor (VIF) was determined to be 1, it may be deduced that the regression analysis contains a considerable level of multicollinearity [32–34]. Out of the five criteria that were screened, only three were found to form important influential factors when subjected to a two-level factor analysis. This was determined based on the results that were obtained.

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

	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	8.46	1	0.0479	8.36	8.56	
A-pH	0.2951	1	0.0500	0.1920	0.3982	1.00
B-Temperature	0.1398	1	0.0500	0.0367	0.2429	1.00
C-Acrylamide	0.2416	1	0.0485	0.1416	0.3416	1.00
D-Glucose	0.0496	1	0.0500	-0.0535	0.1527	1.00
E-Incubation time	0.1964	1	0.0500	0.0933	0.2995	1.00
AC	-0.1695	1	0.0500	-0.2726	-0.0664	1.00
BD	0.0648	1	0.0500	-0.0383	0.1678	1.00
BE	0.0882	1	0.0500	-0.0149	0.1913	1.00
DE	0.0504	1	0.0500	-0.0527	0.1535	1.00
BDE	0.1151	1	0.0500	0.0120	0.2182	1.00

It is clear from the Pareto charts that were constructed for the assessment of each response coefficient for its statistical significance, and which are depicted in **Fig. 1** that this is the case. The t-limit line and the Bonferroni 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. The Bonferroni limit line has a t-value of effect of 3.491, while the t-limit line has a value of 3.49 (t-value of effect: 2.048).

When determining the significance of coefficients, there are three distinct categories that can be used. A-pH, C-Acrylamide, and E-Incubation were the coefficients that were considered to be the most significant since they were the first ones to have a t-value of effect that was more than the limit that Bonferroni had established. B-Temperature and D-Glucose are the factors that fall into this range. 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. This range includes all of the interacting factors, which fell into this range. These findings are consistent with what was discovered by the application of the coefficient estimate.

In the process of developing this bacterium dependent on acrylamide, the most important contributing criteria were the concentration of acrylamide, the pH, and the length of the incubation period. These are qualities that have been identified to be significant in contributing high growth of microbes on acrylamide by several OFAT-based methodologies as being discovered as being crucial contributors. The acrylamide concentrations that were used in this study were well within the range that was known to be tolerated by the majority of bacteria that are capable of degrading acrylamide. Concentrations of acrylamide that are greater than 1000 mg/L are typically toxic to the bacteria that degrade acrylamide. [16–25,35–41].

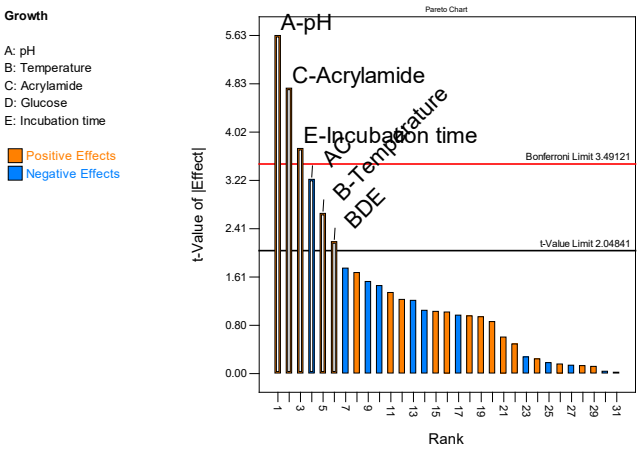

Fig. 1. Pareto chart of operational parameters.

The comparative influence of all of the operational factors is displayed in the model's perturbation plot (**Fig. 2**) at a specific location in the design space. It is clear by examining the plot that the factor A-pH has the steepest slope, followed by the factors C, E, D, and B in that order. The perturbation plot illustrates the fact that the components do interact with one another. The Plackett-Burman screening approach is not equipped to identify interacting effects, which is a significant accomplishment [42–45].

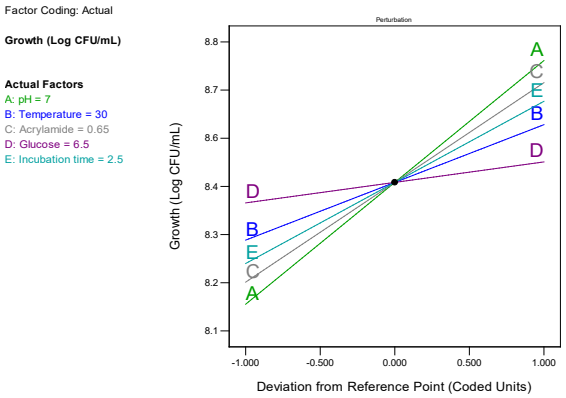

Fig. 2. Perturbation plot of operational parameters obtained through regular two-factor design.

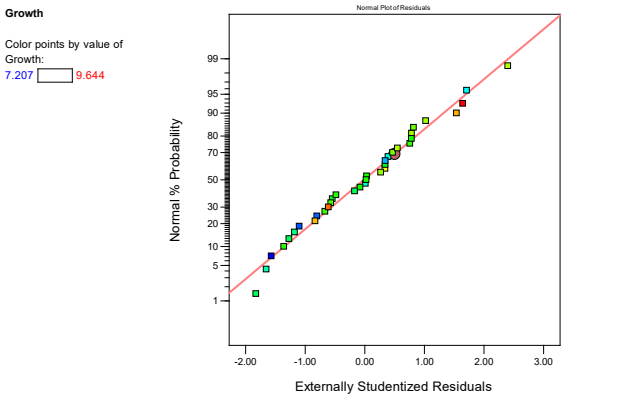

Fig. 3. Diagnostic's plot in the form of the normal plot of residuals for the two level factorial optimization studies.

In this regard, a half-normal probability plot of the residuals was constructed and analyzed (shown as **Fig. 3**), with the goal of determining whether or not the normality assumption was correct. All of the internally studentized residuals values were found to be within 2 and along the straight line, which is evidence that a transformation of the response is not necessary in any way. This was found out while conducting research. As can be seen in **Fig. 4**, the graph that compares the actual experimental findings to the values predicted by the model suggests that there is a strong fit. This may be attributed to the fact that there is a strong correlation between the two sets of data. The Box–Cox plot, which can be found in **Fig. 5**, provides useful direction for selecting the right power law transformation based on the value of lambda.

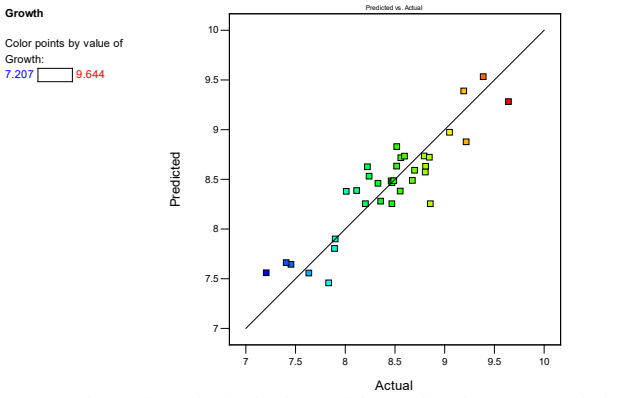

Fig. 4. Diagnostic's plot in the form of the predicted versus actual plot for the two-level factorial optimization studies.

It is not recommended that any additional transformations be made to the observed response in order to fit the model because of the fact that the confidence interval for 95 percent has a value of 1 that corresponds to the value that was designed into the model. This is because the value that was designed into the model was 1. The plot of leverages vs run displayed in **Fig. 6** demonstrates that all of the obtained numerical values lie within the normal limits range of 0–1 and that this is consistent with previous findings. This suggests that it is possible for a design point to have an effect on the way the model fits. In the event that there is a problem with the data point, such as an error that was not anticipated, a high leverage point value of more than one is regarded as "bad" because the error has a large influence on the model. The plot of leverages versus runs reveals that there are no data that have a leverage that is higher than the average leverage. This makes sense given that data with leverages that are higher than this would have an effect on at least one model parameter.

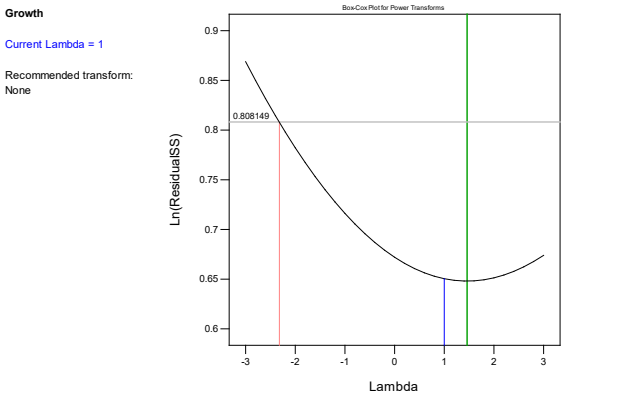

Fig. 5. Diagnostic's plot in the form of Box-Cox plot for the two level factorial optimization studies.

The plot of Cook's distances can be used to produce a measurement of the response outlier that is comparable to an experimental trial (**Fig. 7**). Cook's distances are numbers that cannot be negative, and the higher these values are, the more significant an observation is. An increase in Cook's distances indicates an increase in the significance of an observation. The majority of researchers agree that applying three times the average value of Cook's D to a dataset is the appropriate cutoff point to use for deciding whether or not an observation should be regarded as significant. It has been determined that the values of the Cook's distances are all within a value of 1, and the diagnostic does not prescribe any particular transformation procedures. There are no indications of serial correlation visible in the plot of the residuals versus the run data (**Fig. 8**), which leads one to think that the data is random in terms of the qualities it exhibits [26,27,30,46,47].

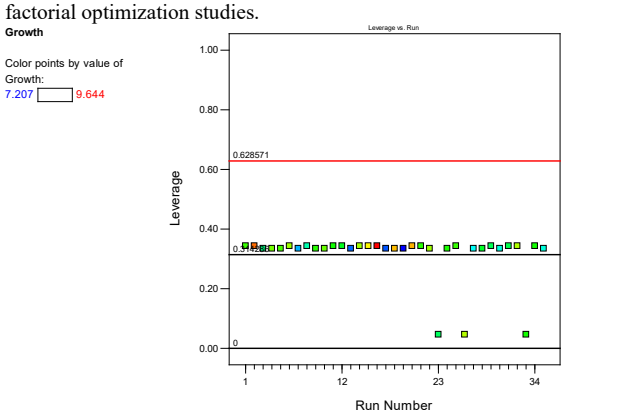

Fig. 6. Diagnostic's plot in the form of leverage vs runs for the two level factorial optimization studies.

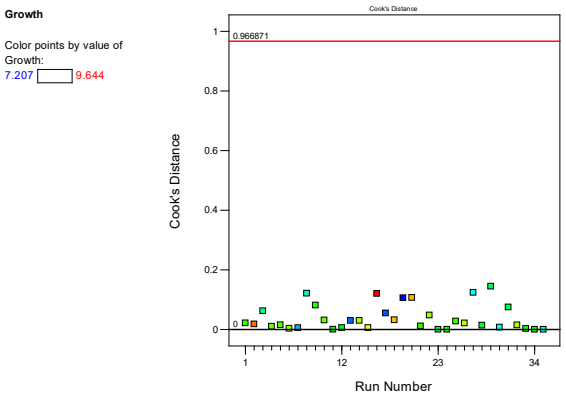

Fig. 7. Diagnostic's plot in the form of Cook's distance vs runs for the two-level factorial optimization studies.

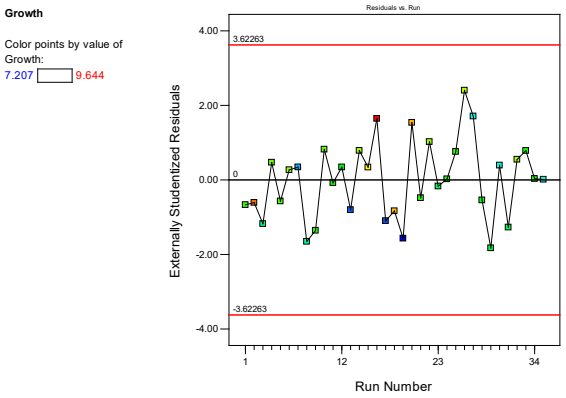

Fig. 8. Diagnostic's plot in the form of residuals vs runs for the two-level factorial optimization studies.

When influential points are brought up, it is not necessarily a problem; nonetheless, it is vital to follow up on observations that are characterized as particularly significant. A high result on an influence measure may indicate a number of different things, including a mistake in the process of data input or an observation that is clearly not typical of the population of interest and therefore needs to be excluded from the analysis. Both of these instances should be taken into consideration when determining whether or not to include the result in the analysis. During the process of fitting a model, the inclusion of one or more data items that are sufficiently relevant has the potential to cause coefficient estimations to be thrown off, which in turn can make it difficult to understand what the model is trying to say.

Histograms and scatterplots were the traditional methods that were utilized in the past in order to determine whether or not a dataset contained any potential outliers prior to carrying out a linear regression. Before beginning the linear regression, this step was performed. Both methods of analyzing data points were subjective, and there was little that could be done to determine the degree to which each potential outlier affected the data that was represented the outcomes. As a direct consequence of this, a variety of quantitative indicators, such as DFFIT and DFBETA, came into being. The DFFFITS method performs an analysis to determine the degree to which each specific sample contributes to the value that was anticipated. According to Cook, it is possible to convert it to the distance being travelled. In contrast to Cook's distances, DFFITS can either have a positive or a negative value depending on the situation. If the number displayed is "0," then the point in question lies exactly on the regression line. Leverage is what enables us to accomplish this. In mathematical terms, it refers to the disparity between the expected value after observation and the predicted value before observation. DFFITS is the externally studentized residual (ti), with strong leverage points multiplied by it and low leverage points lowering it, according to the alternative formula [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**).

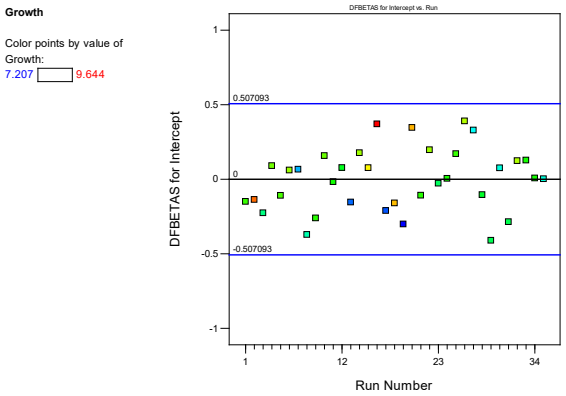

Fig. 9. Diagnostic's plot in the form of DFBETAS for intercept vs runs for the two level factorial optimization studies.

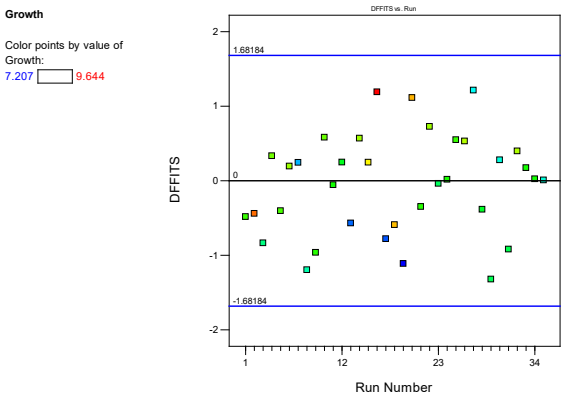

Fig. 10. Diagnostic's plot in the form of DFFITS vs runs for the two-level factorial optimization studies.

CONCLUSION

The screening of five independent parameters that influence the development of the bacterium on acrylamide was carried out using the two-level factorial design. These elements are as follows: pH, temperature, length of incubation period, concentration of acrylamide, and concentration of glucose. The two factor factorial design was successful in finding important contributing parameters in the growth of this bacterium on acrylamide. These important contributing parameters were acrylamide concentration, pH, and incubation time, all of which have the potential to be further optimized by RSM in subsequent works. An analysis of variance (ANOVA), a Pareto chart, a pertubation plot, and several other diagnostic plots were utilized in order to investigate the significant factors or parameters that contributed. The two-level factorial conclusion was supported by diagnostic plots such as half-normal, Cook's distance, residual vs runs, leverage vs runs, Box-Cox, DFFITS, and DFBETAS. The acrylamide concentrations used in this investigation were all well within the range that previous research has shown the majority of acrylamide-degrading bacteria to be able to tolerate. A longer incubation time allows for higher growth, and an incubation time ranging from two to five days for optimal development has been

recorded in several acrylamide-degrading microorganisms. Therefore, the results of incubation time are something that should be predicted. The majority of microorganisms that degrade acrylamide thrive in conditions that are close to neutral, which is consistent with the findings of our study and the trends that have been found in the published literature.

REFERENCES

1. Spencer P, Schaumburg HH. Nervous system degeneration produced by acrylamide monomer. Environ Health Perspect. 1975 Jun 1;11:129–33.
2. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem. 2002;50(17):4998–5006.
3. Tyl RW, Friedman MA. Effects of acrylamide on rodent reproductive performance. Reprod Toxicol. 2003 Jan 1;17(1):1–13.
4. Yang HJ, Lee SH, Jin Y, Choi JH, Han CH, Lee MH. Genotoxicity and toxicological effects of acrylamide on reproductive system in male rats. J Vet Sci. 2005 Jun;6(2):103–9.
5. Backer LC, Dearfield KL, Erexson GL, Campbell JA, Westbrook-Collins B, Allen JW. The effects of acrylamide on mouse germ-line and somatic cell chromosomes. Environ Mol Mutagen. 1989;13(3):218–26.
6. Mottram, DS, Wedzicha BL, Dobson AT. Acrylamide is formed in the Maillard reaction. Nature. 2002;419:448–9.
7. Zamora R, Delgado RM, Hidalgo FJ. Strecker aldehydes and □-keto acids, produced by carbonyl-amine reactions, contribute to the formation of acrylamide. Food Chem. 2011;128(2):465–70.
8. Shukor MY, Gusmanizar N, Azmi NA, Hamid M, Ramli J, Shamaan NA, et al. Isolation and characterization of an acrylamide-degrading *Bacillus cereus*. J Enviromental Biol. 2009;30(1):57–64.
9. Hagmar L, Törnqvist M, Nordander C, Rosén I, Bruze M, Kautiainen A, et al. Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Health. 2001;27(4):219–26.
10. Igisu H, Goto I, Kawamura Y, Kato M, Izumi K. Acrylamide encephaloneuropathy due to well water pollution. J Neurol Neurosurg Psychiatry. 1975;38(6):581–4.
11. Eikmann T, Herr C. How dangerous is actually acrylamide exposure for the population. Umweltmed Forsch Prax. 2002;7(6):307–8.
12. Pruser KN, Flynn NE. Acrylamide in health and disease. Front Biosci - Sch. 2011;3 S(1):41–51.
13. Pennisi M, Malaguarnera G, Puglisi V, Vinciguerra L, Vacante M, Malaguarnera M. Neurotoxicity of acrylamide in exposed workers. Int J Environ Res Public Health. 2013;10(9):3843–54.
14. Rahim MBH, Syed MA, Shukor MY. Isolation and characterization of an acrylamide-degrading yeast *Rhodotorula* sp . strain MBH23 KCTC 11960BP. J Basic Microbiol. 2012;52(5):573–81.
15. Wakaizumi M, Yamamoto H, Fujimoto N, Ozeki K. Acrylamide degradation by filamentous fungi used in food and beverage industries. J Biosci Bioeng. 2009;108(5):391–3.
16. Wampler DA, Ensign SA. Photoheterotrophic metabolism of acrylamide by a newly isolated strain of Rhodopseudomonas palustris. Appl Environ Microbiol. 2005;71(10):5850–7.
17. Buranasilp K, Charoenpanich J. Biodegradation of acrylamide by *Enterobacter aerogenes* isolated from wastewater in Thailand. J Environ Sci. 2011;23(3):396–403.
18. Charoenpanich J, Tani A. Proteome analysis of acrylamide-induced proteins in a novel acrylamide-degrader *Enterobacter aerogenes* by 2D electrophoresis and MALDI-TOF-MS. Chiang Mai Univ J Nat Sci. 2014;13(1):11–22.
19. Gusmanizar N, Shukor Y, Ramli J, Syed MA. Isolation and characterization of an acrylamide-degrading *Burkholderia* sp. strain DR.Y27. J Ris Kim. 2015 Feb 11;2(1):34.
20. Yu F, Fu R, Xie Y, Chen W. Isolation and characterization of polyacrylamide-degrading bacteria from dewatered sludge. Int J Environ Res Public Health. 2015;12(4):4214–30.
21. Bedade DK, Singhal RS. Biodegradation of acrylamide by a novel isolate, *Cupriavidus oxalaticus* ICTDB921: Identification and characterization of the acrylamidase produced. Bioresour Technol. 2018 Aug 1;261:122–32.
22. Aisami A, Gusmanizar N. Characterization of an acrylamide-degrading bacterium isolated from hydrocarbon sludge. Bioremediation Sci Technol Res. 2019 Dec 28;7(2):15–9.
23. Othman AR, Rahim MBHA. Modelling the Growth Inhibition Kinetics of *Rhodotorula* sp. strain MBH23 (KCTC 11960BP) on Acrylamide. Bioremediation Sci Technol Res. 2019 Dec 28;7(2):20–5.
24. Rusnam, Gusmanizar N. An Acrylamide-degrading Bacterial Consortium Isolated from Volcanic Soil. J Biochem Microbiol Biotechnol. 2021 Dec 31;9(2):19–24.
25. Rusnam, Gusmanizar N. Characterization of An Acrylamide-degrading Bacterium Isolated from Volcanic Soil. J Environ Bioremediation Toxicol. 2022 Aug 5;5(1):32–7.
26. Hedayat AS, Pesotan H. Designs for two-level factorial experiments with linear models containing main effects and selected two-factor interactions. J Stat Plan Inference. 1997;64(1):109–24.
27. Leitnaker MG, Mee RW. Analytic use of two-level factorials in incomplete blocks to examine the stability of factor effects. Qual Eng. 2001;14(1):49-58+xii.
28. Chang FK, Ting CP. Optimal two-level fractional factorial designs for location main effects with dispersion factors. Commun Stat - Theory Methods. 2011;40(11):2035–43.
29. Besseris GJ. Order statistics for the two-level eight-run saturated-unreplicated fractional-factorial screening: Corrections for asymmetry in the three-and four-factor multi-contrasting. Int J Qual Eng Technol. 2013;3(3):236–42.
30. Parui S, Parsad R, Mandal BN. Efficient block designs for incomplete factorial experiments for two factors with unequal block sizes. Commun Stat - Theory Methods. 2021;50(11):2531–45.
31. Haida Z, Ab Ghani S, Juju Nakasha J, Hakiman M. Determination of experimental domain factors of polyphenols, phenolic acids and flavonoids of lemon (Citrus limon) peel using two-level factorial design: Determination of experimental domain factors. Saudi J Biol Sci. 2022;29(1):574–82.
32. Bonate PL. Linear Models and Regression. In: Bonate PL, editor. Pharmacokinetic-Pharmacodynamic Modeling and Simulation [Internet]. Boston, MA: Springer US; 2011 [cited 2022 Dec 24]. p. 61–100. Available from: https://doi.org/10.1007/978-1-4419-9485-1_2
33. Mundry R. From nonparametric tests to mixed models: A brief overview of statistical tools frequently used in comparative psychology. In: APA handbook of comparative psychology: Basic concepts, methods, neural substrate, and behavior, Vol 1. Washington, DC, US: American Psychological Association; 2017. p. 157–77. (APA handbooks in psychology®).
34. Fox J. Regression Diagnostics: An Introduction. SAGE Publications; 2019. 138 p.
35. Shah M. Microbial Degradation of Acrylamide by Enterobacter spp. Am J Water Resour. 2014 Nov 2;2:134–40.
36. Shimamura Y, Yui T, Horiike H, Masuda S. Toxicity of combined exposure to acrylamide and Staphylococcus aureus. Toxicol Rep. 2022 Jan 1;9:876–82.
37. Kulkarni NH, Muley AB, Bedade DK, Singhal RS. Cross-linked enzyme aggregates of arylamidase from Cupriavidus oxalaticus ICTDB921: process optimization, characterization, and application for mitigation of acrylamide in industrial wastewater. Bioprocess Biosystem Eng.. 2020 Mar;43(3):457-71
38. Lakshmikandan M, Sivaraman K, Elaiya Raja S, Vasanthakumar P, Rajesh RP, Sowparthani K, et al. Biodegradation of acrylamide by acrylamidase from Stenotrophomonas acidaminiphila MSU12 and analysis of degradation products by MALDI-TOF and HPLC. Int Biodeterior Biodegrad. 2014;94:214–21.
39. Chandrashekar V, Chandrashekar C, Shivakumar R, Bhattacharya S, Das A, Gouda B, et al. Assessment of acrylamide degradation potential of *Pseudomonas aeruginosa* BAC-6 isolated from industrial effluent. Appl Biochem Biotechnol. 2014;173(5):1135–44.
40. Emmanuel Joshua Jebasingh S, Lakshmikandan M, Rajesh RP, Raja P. Biodegradation of acrylamide and purification of acrylamidase from newly isolated bacterium *Moraxella osloensis* MSU11. Int Biodeterior Biodegrad. 2013;85:120–5.
41. Ansede JH, Pellechia PJ, Yoch DC. Metabolism of acrylate to beta-hydroxypropionate and its role in dimethylsulfoniopropionate lyase

induction by a salt marsh sediment bacterium, Alcaligenes faecalis M3A. Appl Environ Microbiol. 1999 Nov;65(11):5075–81.
42. Dey A, Suen CY, Das A. Asymmetric fractional factorial plans optimal for main effects and specified two-factor interactions. Stat Sin. 2005;15(3):751–65.
43. Bailey RA, Łacka A. Nested row-column designs for near-factorial experiments with two treatment factors and one control treatment. J Stat Plan Inference. 2015;165:63–77.
44. Lawson J. Comparison of conditional main effects analysis to the analysis of follow-up experiments for separating confounded two-factor interaction effects in 2 IVk−p fractional factorial experiments. Qual Reliab Eng Int. 2020;36(4):1454–72.
45. Chobisa D. Design of Experiments for the Development of Injectable Drug Products. In: Beg S, editor. Design of Experiments for Pharmaceutical Product Development: Volume II : Applications and Practical Case studies. Singapore: Springer; 2021 [cited 2022 Dec 24]. p. 69–96. Available from: https://doi.org/10.1007/978-981-33-4351-1_5
46. Draper NR, Stoneman DM. Factor Changes and Linear Trends in Eight-Run Two-Level Factorial Designs. Technometrics. 1968;10(2):301–11.
47. Wei J, Carroll RJ, Harden KK, Wu G. Comparisons of treatment means when factors do not interact in two-factorial studies. Amino Acids. 2012;42(5):2031–5.
48. Cornell JA. Classical and Modern Regression With Applications. Technometrics. 1987 Aug 1;29(3):377–8.
49. Aydin D, Alma ÖG. Diagnostics of influential observations in partially linear models. Erzincan Univ J Sci Technol. 2015 Jan 2;8(1):51–68.