

# Modelling Effects of Process Variables During Fermentation of Pineapple Peels Using Yeast for Ethanol Production Using a Second Order Optimal Rotatable Design in Four Dimensions.

Kabue Timothy Gichuki<sup>1\*</sup>, Koske Joseph<sup>2</sup>, Mutiso John<sup>2</sup>

1. School of Pure and Applied Sciences, Mount Kenya University, Thika Kenya

2. School of Sciences and Aerospace Studies, Moi University Eldoret, Kenya

\*Email Address: [kabuegichuki@yahoo.com](mailto:kabuegichuki@yahoo.com)

## Abstract

The need for a cleaner environment in urban areas and the high cost of petroleum products which are becoming scarce due to unbalanced relation between supply and demand besides air pollution of sources has led to the research for other fuels to replace fossil fuels. Ethanol from biomass waste is such an alternative to petroleum products. Most studies on optimization of process variables using Response Surface Methodology apply Central Composite Designs yet other designs exist. Optimal designs have fewer trials employed with the aim of obtaining efficient designs for fitting reduced quadratic or higher order models. Coded values of a second order optimal rotatable design in four dimensions constructed using balanced incomplete block designs (BIBD) was fit into experimental data in order to study the effects of four process variables namely; time, PH, temperature and substrate concentration on fermentation of pineapples peels using *Saccharomyces cerevisiae* for ethanol production. Normal probability plots and Multiple R-squared of 0.9323 and Adjusted R-squared of 0.8944 which measure model fitting reliability indicated aptness of the model. Most values of Probability F were less than 0.05, confirming that the model terms were significant and only 6.8% of the total variation could not be explained by the model ensuring good adjustment of the model to experimental data. Model adequacy was also confirmed by the good agreement between the experimental data and predicted values. The design was found reliable in modeling, and studying the effects of the four factors to the processes of fermentation of pineapples peels as substrate for ethanol production using *Saccharomyces cerevisiae*

**Keywords:** *Ethanol, Pineapple Peels, Response Surface Methodology and Rotatable Designs.*

## 1. Introduction

Ethanol from bio-mass waste is an alternative to fossil fuels that can be used in petrol engines without modification and with the current fueling infrastructure and it is easily applicable in present day combustion engine, as mixing with gasoline Hansen et al.,(2005b). Combustion of ethanol results in relatively low emission of volatile organic compounds, carbon monoxide and nitrogen oxides compared to fossil fuels such as petrol and diesel Wyman and Hinman, (1990). Isaias et al., (2004) observed that ethanol reduces green-house gases by between 86% to 90% while Goettemoeller and Goettemoeller, (2007) noted that many starchy wastes can be used as raw materials for ethanol production but Molasses is widely used since it is cheap and readily available for conversion with little pre-treatments as compared to other starchy materials since it's sugars are present in fermentable form, Razmovski and Vucurovic, (2011). Tropea et al (2014) notes that 75% of the fruit processed in canneries results in peeled skin, core, and crown as the end waste products, which are rich in intracellular sugars and plant cell walls composed mainly of cellulose, peptic substances and hemicelluloses. Their dry matter content which is around 10%, is composed of about 96% organic and 4% inorganic matter Abdullah, (2007). These materials exhibit both high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values as noted by Ban-Koffi and Han, (1990) which give rise to serious pollution problems if not properly disposed. For high quality and high yield of ethanol in ethanol industry, selection of fermentative yeast is key. *Saccharomyces cerevisiae* is the most well-known and commercially significant yeasts that have been primarily used for bioethanol production Chandel et al., (2007). Tsuyoshi et al., (2005), records that one yeast cell ferments approximately its own weight of glucose per hour and that Sugars from sugar cane, sugar beets, molasses, and fruits are converted to ethanol directly. Choonut et al., (2014) observed that Pineapple peels, account for 29-40% (w/w) of total pineapple weight which after pretreatment with water and heat at 100<sup>0</sup>C for four hours, 36.25±2.87% of cellulose

is achieved. A number of factors like incubation time and temperature, substrate concentration and sugar tolerance of the yeast strain used in fermentation are known to limit the production of ethanol in quality and quantity. Use of concentrated sugar substrate is one way of obtaining high ethanol yield during fermentation, but high substrate concentrations is inhibitory to fermentation due to the osmotic stress on yeast. While certain ranges of pH and temperatures of the fermentation medium significantly affect the process. Ergun et al.,(2000) and Jones et al.,(1981).

### 1.1. Response Surface Methodology (RSM)

Introduced by Box and Wilson,(1951),RSM is useful for modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response Montgomery, (2005) , by finding the most appropriate production conditions for the bioprocess and forecasting response Isaias *et al.*, (2004). RSM is concerned with the selection and construction of an appropriate design that can provide adequate and reliable information concerning a certain response variable, denoted by Y and determination of a suitable model that best fits the data that can be generated from using the design chosen. Such a model gives an approximate functional relationship between the response variable Y and a set of control variables denoted by  $x_i$  which are believed by the experimenter to have an effect on the response. Then finally the determination of optimal settings on the control variables that produce maximum (or minimum) response values within a certain region of interest R.

$$y_u = f(x_{iu}) + e_u \quad (1)$$

Where,  $u = 1, 2, \dots, N$  are the  $N$  observations and  $x_{iu}$  is the level of the  $i^{th}$  factor at the  $u^{th}$  run. The function describes the form in which the response and input variables are related and  $e_u$  is the experimental error at the  $u^{th}$  run with mean zero ( $\mu = 0$ ) and variance  $\sigma^2$ . The objective is to establish a functional relationship between the response and the control variables which give a summary of an experiment and enables prediction of response  $y_u$  for values of  $x_{iu}$  that are not included in the experiment.

### 1.2 Rotatable Designs

Introduced by Box and Hunter, (1957) for the exploration of response surface where it was shown that the number of Centre points in the rotatable central composite designs could be chosen to provide a design with uniform precision for the estimated surface within one unit of the design center co-ordinates on the coded scale since the interest is in the response surface near the Centre of the design. Tables of designs or methods of construction of these designs can be found in Draper, (1985). Most of these designs are based on  $2^{n-p}$  fractional factorial designs augmented with design center points to estimate second-order response surface models. Das and Narasimham, (1962) demonstrated construction of second and third order rotatable designs using balanced incomplete block designs (BIBD) while Koske, Kosgei, and Mutiso, (2011) developed a third order rotatable design in five dimensions with 320 points through balanced incomplete block design.

## 2. Materials and Method

Pineapple peels were obtained from a local market in Thika town. The peels were sun-dried for three days and then oven dried at 35°C for three hours and milled into fine particles using a Ramtons fruit blender and 40g of the mill was carefully weighed (using an electronic balance (type AY220 number D440620174 of capacity 220g with a reliability index of 0.1mg manufactured by Shimadzu Corporation) and dissolved in 1000mL of distilled water and immediately pre-treated at 100 °C for 240 minutes under continuous mixing to inactivate endogenous enzymes and reduce microbial spoilage. The content was cooled to room temperature. The filtrate settled at the bottom of the glass jar, a pH of 3.95 was observed using a pH meter model number LMPH-10 Mark LABMAN serial number L9254.

### 2.1 Preparation of the Reagents

Acidified potassium dichromate (0.01M in 5.0M sulphuric acid), Starch indicator solution (1.0 % starch solution) ,Potassium iodide 1.2molar and 0.03molar Sodium thiosulfate solution ( $\text{Na}_2\text{S}_2\text{O}_3^- \text{OH}$ ) used in titration were all prepared as described by Ferguson, n.d.

### 2.2 Process Variables of Bioethanol

Gichuki *et al.*, (2020) gave the design matrix using the coded levels for a four factor design when the number of replications ( $r$ ) are less than three the number( $\lambda$ ) of times pairs of treatments occur in the design as put forward by Das and Narasimham, (1962) . This design matrix was used in this study to develop a model for studying the effects of time, initial pH of the fermentation broth, incubation temperature and substrate concentration on ethanol

yield and interaction effects of factors on the amount of ethanol produced. The coded and actual levels of the process variables are displayed in Table 1

Table1. Coded and Actual Levels of the Process Variables.

Coded levels	T(hrs.)	pH	T(°C)	Conc(g/l)
2.116	72	7	40	40
1.137	60.9	6.3	36.5	35.4
0	48	5.5	32.5	30
-1.137	35.1	4.7	28.5	24.6
-2.116	24	4	25	20

The factors were rescaled and therefore zero is in the middle of the center of the design while  $\pm 1.137$  and  $\pm 2.116$  are the distances from the center with directions which correspond to the factorial and axial parts of the design respectively. The coding of  $x_i$ 's substantially increases the flexibility of the model Hill and Hunter, (1966). The natural variables  $x_i$  for  $i = 1,2,3,4$  factors were converted into coded variables using the following relationship;

$$x_i = \frac{\xi_i - [\max x_i + \min x_i] / 2}{[\max x_i - \min x_i] / (2.116 * 2)} \quad (2)$$

The maximum and minimum values of  $x_i$  cover the range of variation in the input variables where  $\xi_i$  represents the natural variable. The output response corresponding to each combination of input parameters was the mean Bioethanol produced in g/L per experimental run. The pH levels were adjusted using sodium hydroxide.

### 2.3 Fermentation Process Parameters

Fermentations was carried out in 250 ml conical flasks fitted with rubber stoppers and clearly marked with stickers indicating run number, required fermentation time, pH, temperature and concentration of substrate. Five batches of 250 ml in conical flasks of the pre-treated substrate were prepared and concentrations varied by diluting using distilled water, further five other 100mL beakers were used where substrates with various concentration were placed and pH adjusted by adding drops of 0.1M Sodium hydroxide to increase it from 3.95 which was found to be the pH of the pre-treated substrate. Then 10ml of these substrates with concentrations and pH's adjusted as per the experimental design points were drawn using a Pipette and placed in the marked 250ml conical flasks of the 40 experimental runs. The fermentation started with addition of 10 ml of Saccharomyces Cerevisiae inoculum prepared by dissolving 5g of Brewer's yeast in 1000mL of distilled water to the medium. The contents were placed in a rotation shaker at 30 rpm for two hours. Incubation was performed in four shaking incubators at 200rpm in Mount Kenya University analytical chemistry laboratories set at different temperatures as per the design requirements and samples for analysis were taken after 24, 35.1,48,60.9 and 72hours at different prescribed incubation periods. Broth samples were drawn from the fermentation flasks using a 10 ml syringe: The drawn samples were immediately frozen at -10 °C in a deep freezer until analysis time.

### 2.4 Ethanol Determination

Ferguson, n.d. method was applied where samples from each experimental run were diluted in the ratio of 1:10. Then three samples of 1ml of the diluted sample were drawn using a micro Pippete and placed in a 5ml glass vial and 10ml of the acid dichromate solution was transferred to a 250ml conical flasks with matching rubber stoppers and the fermented samples were suspended over the dichromate for overnight. Three samples from each experimental run were prepared since the entire content of the conical flask was used in the titration. Then water and ethanol from the sample slowly evaporated from the glass vial and ethanol was oxidized to ethanoic acid by the dichromate, the set up was left in a water bath at 25°C – 30°C degrees. Analysis of ethanol content in the sample was by the method of redox back titration as put forward by Krakowiak, et al.,(1997) where for sharp end points detections during titrations, dilution of the fermented samples in the ratio of one is to ten was necessary. According to Ferguson, n.d., ethanol is oxidized to acetaldehyde by acid reacting it with an excess of acidified Potassium dichromate. The excess potassium dichromate is oxidized by potassium iodide to produce iodine then iodine produced is titrated with standard sodium thiosulfate solution. Three concordant results (titers agreeing to within 0.1ml) were obtained Three blank titrations were carried out to inform on amount of acid dichromate present at the start as no alcohol had been added it meant all dichromate was still present.

### 2.5 Calculation of Ethanol Content in the Samples

The average volume of sodium thiosulfate used in titration of the sample is subtracted from the average volume of sodium thiosulfate used in titrating the blank and the number of moles of sodium thiosulfate in this volume obtained: Six moles of  $Na_2SO_3^{2-}$  is equivalent to one mole of  $Cr_2O_7^{2-}$  and two moles of  $Cr_2O_7^{2-}$  is equivalent to three moles of ethanol and one mole of  $Na_2SO_3^{2-}$  is equivalent to 0.25moles of ethanol.

### 3. Results and discussions

The fitted full second order model using R programming is

$$\hat{y} = 11.82 + 0.513X_1 - 0.269X_2 + 0.994X_3 + 0.109X_4 - 0.759X_1X_2 + 0.865X_1X_3 + 0.213X_1X_4 + 0.445X_2X_3 + 1.117X_2X_4 - 0.372X_3X_4 - 1.03X_1^2 - 0.627X_2^2 - 1.210X_3^2 - 1.138X_4^2 \quad (3)$$

Equation (3) characterizes the influence of the different variables on yield. Positive signs in front of the terms indicate synergetic effect while negative sign indicate antagonistic effects. Within the studied range of the variables, pH ( $X_2$ ), had an antagonistic effect on yield of 0.269 while all other three variables had synergetic effect with temperature having the highest effect of 0.994. A comparison of the observed results between experimental and predicted values showed a good matching in between and over the defined range.

#### 3.1 Plot of Residuals versus Fitted Values

If the model is correct, and assumptions of normality for the errors are satisfied, residuals should be structureless, in particular they should be unrelated to any other variables including the response and the predicted values. Plot of the residuals versus fitted values should not reveal any obvious pattern. The normal probability plot, of the residuals are shown in figure 1.

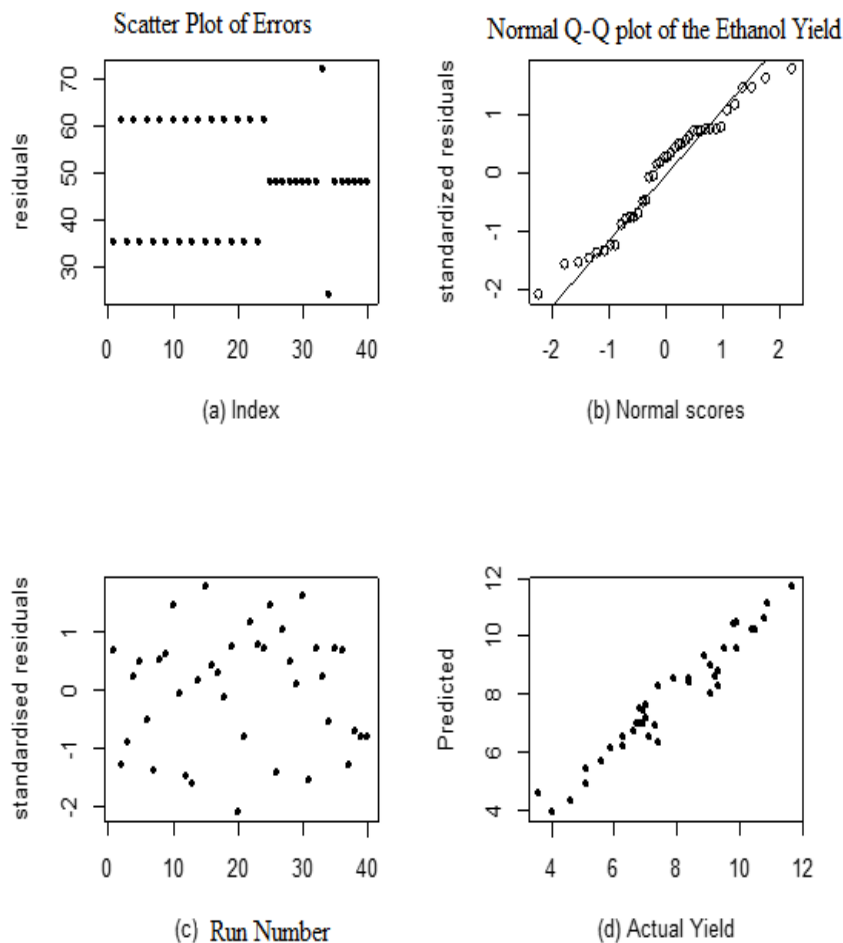


Figure 1. Plots of Residuals

In figure 1(a) is the scatter plot of the errors while (b) is the Normal Q-Q plot of ethanol yield which clearly approximates a straight line. In Figure 1(c), the standardized residuals plotted against the run numbers shows how randomly they are scattered within the constant range of residuals across the graph thus the model is adequate and finally the plot of predicted values against the experimental values figure 1(d) indicates a strong positive correlation hence no reason to suspect violation of independence or constant variance assumption.

### 3.2 Anova and Regression Analysis

Analysis of the fitted model to test whether the model adequately approximates bioethanol yield well was carried out Using the Regression and ANOVA Table 2 and Table 3 respectively.

Table 2. Call: Rsm (Formula = Y ~ So (X1, X2, X3, X4)

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	11.82132	1.71799	6.8809	3.266e-07 ***
X1	0.51267	0.10282	4.9861	3.864e-05 ***
X2	-0.26866	0.10282	-2.6129	0.014978 *
X3	0.99367	0.10282	9.6641	6.360e-10 ***
X4	0.10893	0.10282	1.0594	0.299524
X1:X2	-0.75903	0.12573	-6.0371	2.628e-06 ***
X1:X3	0.86539	0.12573	6.8831	3.249e-07 ***
X1:X4	0.21272	0.12573	1.6919	0.103088
X2:X3	0.44478	0.12573	3.5377	0.001606 **
X2:X4	1.11679	0.12573	8.8827	3.311e-09 ***
X3:X4	-0.37226	0.12573	-2.9609	0.006632 **
X11	-1.02999	0.43578	-2.3635	0.026185 *
X22	-0.62737	0.43578	-1.4396	0.162377
X33	-1.21016	0.43578	-2.7770	0.010248 *
X44	-1.13815	0.43578	-2.6117	0.015017 *

--Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1. Multiple R-squared: 0.9323, Adjusted R-squared: 0.8944 F-statistic: 24.59 on 14 and 25 DF, p-value: 3.692e-11. Call: F-value of 24.59 and the low probability value (< 0.001), is proof of the significant model fit. Using the  $t$ -test statistics and 5% level of significance, the regression analysis indicates that out of the four control variables, incubation time ( $X_1$ ) and temperature ( $X_3$ ) were significant to the yield with their  $P$ -values of their  $t$ -statistics being far much less than 0.001 the level of significance while initial PH ( $X_2$ ) had a significant negative effect at  $P$ -value of 0.01 while the concentration of the substrate was not significant at  $\alpha = 5\%$ . The interactive effect of temperature and time, PH and substrate concentration had very significant synergetic effect on bioethanol yield while time and pH had an antagonistic effect significant at 0.001. Interactive effect of temperature and pH was only significant at 0.01 implying that increase in both incubation temperature and initial PH resulted in increase in ethanol yield. The intercept term of the model was significant at P-value of 0.001. While the quadratic effects of time, temperature and substrate concentration were all significant at a P-value of 0.05 affecting bioethanol production antagonistically. The  $t$ -statistics values could also have been determined analytically as in equation (4)

$$t_0 = \frac{\hat{\beta}_1}{\sqrt{\sigma^2 w_{jj}}} \quad (4)$$

Where  $w_{jj}$  is the diagonal element of matrix  $(X'X)^{-1}$  corresponding to  $\hat{\beta}_1$ . The denominator of equation (4) being standard error of coefficient  $\hat{\beta}_1$ . For example

$$t_{X_1} = 4.986142 \quad (5)$$

The reduced model after removing the non-significant terms became

$$\hat{Y} = 11.82 + 0.51X_1 - 0.27X_2 + 0.99X_3 + 0.11X_4 - 0.76X_1X_2 + 0.87X_1X_3 + 0.44X_2X_3 + 1.12X_2X_4 - 0.37X_3X_4 - 1.03X_1^2 - 1.21X_3^2 - 1.14X_4^2 \quad (6)$$

Equation (6) was used to obtain the estimated value of ethanol yield in Table 4. To test the adequacy of the fitted model, analysis of variance and F-test were employed. The Analysis of variance table for full second order model is given in Table 3.

Table 3: Analysis of Variance

Response: Y	D.O.F	Sum SQ	Mean SQ	F value	Pr (>F)
FO(X1, X2, X3, X4)	4	53.346	13.3364	31.5516	1.940e-09
TWI(X1, X2, X3, X4)	6	78.988	13.1646	31.1451	1.944e-10
PQ(X1, X2, X3, X4)	4	13.157	3.2894	7.7821	0.0003228
Residuals		25	10.567	0.4227	
Lack of fit		25	10.567	0.4227	
Pure error	0	0.000			

The F-ratio for main effects; time, pH, temperature and substrate concentration of 31.5516 is more than table F(5%,4,35)=2.69. Hence reject the null hypothesis of equality of the regression parameters at 5%. The two terms interactions and quadratic regression equality of parameters hypotheses were rejected since their F-ratio values of 31.1451 and 7.7821 respectively with P-values 1.944e - 10 and 0.0003228 were both less than  $\alpha = 5\%$ . Since the second order terms of two-way interactions (TWI) and polynomial quadratics (PQ) terms contributed significantly, the model is considered adequate. Multiple R-squared of 0.9323 and Adjusted R-squared of 0.8944 which measure model fitting reliability indicates aptness of the model, Bhunia and Dey, (2012) Also most values of Probability F are less than 0.05, which confirms that the model terms are significant. Only 6.8% of the total variation cannot be explained by the model which ensures good adjustment of the model to experimental data. Model adequacy was also confirmed by the good agreement between the experimental data which ranged between 3.6 to 11.7g/L and predicted values from 4.3 and 12.4g/L as shown in Table 4.

#### 4. Conclusion:

RSM and the rotatable design constructed using balanced incomplete block design was found reliable in modeling, and studying the effects of the four factors to the processes of fermentation of pineapples peels as substrate for ethanol production using *Saccharomyces cerevisiae*. Further studies on modelling the effects of these and other process variables which affect the quantity of ethanol produced using pineapple peels using a rotatable optimal design constructed using balanced incomplete blocks designs when the number of replications are more than three the number of times pairs of treatments occur in the whole design is suggested

Table 4. Observed, Estimated and Residuals Values

Run	X1	X2	X3	X4	Time	pH	Temp	Conc	Y Observed	Y Estimated	Errors
1	-1.137	0	-1.137	-1.137	35.1	5.5	28.5	24.6	7.1	6.3	0.8
2	1.137	0	-1.137	-1.137	60.9	5.5	28.5	24.6	5.1	5.2	-0.1
3	-1.137	0	1.137	-1.137	35.1	5.5	36.5	24.6	6.8	7.2	-0.4
4	1.137	0	1.137	-1.137	60.9	5.5	36.5	24.6	9.8	10.6	-0.8
5	-1.137	0	-1.137	1.137	35.1	5.5	28.5	35.4	7	7.5	-0.5
6	1.137	0	-1.137	1.137	60.9	5.5	28.5	35.4	6.6	6.4	0.2
7	-1.137	0	1.137	1.137	35.1	5.5	36.5	35.4	6.3	6.5	-0.2
8	1.137	0	1.137	1.137	60.9	5.5	36.5	35.4	10.5	9.9	0.6
9	-1.137	-1.137	0	-1.137	35.1	4.7	32.5	24.6	8.4	8.2	0.2
10	1.137	-1.137	0	-1.137	60.9	4.7	32.5	24.6	10.9	11.3	-0.4
11	-1.137	1.137	0	-1.137	35.1	6.3	32.5	24.6	6.7	8.4	-1.7
12	1.137	1.137	0	-1.137	60.9	6.3	32.5	24.6	5.6	7.6	-2
13	-1.137	-1.137	0	1.137	35.1	4.7	32.5	35.4	5.1	7.3	-2.2
14	1.137	-1.137	0	1.137	60.9	4.7	32.5	35.4	9.1	10.4	-1.3
15	-1.137	1.137	0	1.137	35.1	6.3	32.5	35.4	9.9	9.8	0.1
16	1.137	1.137	0	1.137	60.9	6.3	32.5	35.4	8.9	9	-0.1
17	-1.137	-1.137	-1.137	0	35.1	4.7	28.5	30	6.9	7.7	-0.8
18	1.137	-1.137	-1.137	0	60.9	4.7	28.5	30	7.4	8.5	-1.1
19	-1.137	1.137	-1.137	0	35.1	6.3	28.5	30	7	9	-2
20	1.137	1.137	-1.137	0	60.9	6.3	28.5	30	3.6	6	-2.4
21	-1.137	-1.137	1.137	0	35.1	4.7	36.5	30	7.4	7.7	-0.3
22	1.137	-1.137	1.137	0	60.9	4.7	36.5	30	11.7	13	-1.3
23	-1.137	1.137	1.137	0	35.1	6.3	36.5	30	9.3	9	0.3
24	1.137	1.137	1.137	0	60.9	6.3	36.5	30	10.4	10.5	-0.1
25	0	-1.137	-1.137	-1.137	48	4.7	28.5	24.6	9.2	7.9	1.3
26	0	1.137	-1.137	-1.137	48	6.3	28.5	24.6	4	6.2	-2.2
27	0	-1.137	1.137	-1.137	48	4.7	36.5	24.6	10.8	11.1	-0.3
28	0	1.137	1.137	-1.137	48	6.3	36.5	24.6	9.3	9.4	-0.1
29	0	-1.137	-1.137	1.137	48	4.7	28.5	35.4	7.3	8	-0.7
30	0	1.137	-1.137	1.137	48	6.3	28.5	35.4	9.1	8.5	0.6
31	0	-1.137	1.137	1.137	48	4.7	36.5	35.4	6.9	9.3	-2.4
32	0	1.137	1.137	1.137	48	5.5	36.5	35.4	9.9	9.8	0.1
33	2.116	0	0	0	72	5.5	32.5	30	9.3	8.3	1
34	-2.116	0	0	0	24	7	32.5	30	5.9	6.1	-0.2
35	0	2.116	0	0	48	4	32.5	30	8.4	11.2	-2.8
36	0	-2.116	0	0	48	5.5	32.5	30	9.5	12.4	-2.9
37	0	0	2.116	0	48	5.5	40	30	7.9	8.5	-0.6
38	0	0	-2.116	0	48	5.5	25	30	4.6	4.3	0.3
39	0	0	0	2.116	48	5.5	32.5	40	6.8	6.9	-0.1
40	0	0	0	-2.116	48	5.5	32.5	20	6.3	6.5	-0.2

## References

- Abdullah. (2007). SOLID AND LIQUID PINEAPPLE WASTE UTILIZATION FOR LACTIC ACID FERMENTATION. *Reaktor*, 11(1), 50–52.
- Ban-Koffi, L., & Han, Y. W. (1990). Alcohol production from pineapple waste. *World Journal of Microbiology & Biotechnology*, 6(3), 281–284. <https://doi.org/10.1007/BF01201297>
- Bhunja, B., & Dey, A. (2012). Statistical Approach for Optimization of Physiochemical Requirements on Alkaline Protease Production from *Bacillus licheniformis* NCIM 2042. *Enzyme Research*, 2012, 1–13. <https://doi.org/10.1155/2012/905804>
- Box, G. E. ., & Wilson, K. . (1951). On The Experimental Attainment of Optimum Conditions. *Journal Royal Statistics, Soc.*, 13(1), 1-45.
- Box, G. E. P., & Hunter, J. S. (2007). Multi-Factor Experimental Designs for Exploring Response Surfaces. *The Annals of Mathematical Statistics*, 28(1), 195–241. <https://doi.org/10.1214/aoms/1177707047>
- Chandel, A. K., Es, C., Rudravaram, R., Narasu, M. L., Rao, V., & Ravindra, P. (2007). Economics and environmental impact of bioethanol production technologies : an appraisal. *Biotechnology and Molecular Biology Review*, 2(February), 14–32.
- Choonut, A., Saejong, M., & Sangkharak, K. (2014). The Production of Ethanol and Hydrogen from Pineapple Peel by *Saccharomyces cerevisiae* and *Enterobacter aerogenes*. *Energy Procedia*, 52, 242–249. <https://doi.org/10.1016/j.egypro.2014.07.075>
- Das, M., & Narasimham, V. (1962). Construction of rotatable designs through balanced incomplete block designs. *Annals of Mathematical Statistics*, 33(4).
- Draper, R. (1985). *Response Transformation* : 50, 523–525.
- Ergun, M., & Ferda Mutlu, S. (2000). Application of a statistical technique to the production of ethanol from sugar beet molasses by *Saccharomyces cerevisiae*. *Bioresource Technology*, 73(3), 251–255. [https://doi.org/10.1016/S0960-8524\(99\)00140-6](https://doi.org/10.1016/S0960-8524(99)00140-6)
- Ferguson, J. (n.d.). *Estimation of Alcohol Content in Wine by Dichromate Oxidation followed by Redox Titration Chemical Concepts and Techniques : Equipment Required : Reagent Preparation : Method :*
- Gichuki, K. T., Joseph, K., & John, M. (2020). The D-, A-, E- and T-optimal Values of a Second Order Rotatable Design in Four Dimension Constructed Using Balanced Incomplete Block Designs. 8(3), 83–88. <https://doi.org/10.11648/j.ajam.20200803.12>
- Goettemoeller, J., & Goettemoeller, A. (2007). *Sustainable Ethanol: Biofuels, Biorefineries, Cellulosic Biomass, Flex-fuel Vehicles, and Sustainable Farming for Energy Independence*. Michigan: Prairie Oak Pub.
- Hansen, A. C., Zhang, Q., & Lyne, P. W. L. (2005). Ethanol – diesel fuel blends — a review. *Bioresource Technology*, 96(2005), 277–285. <https://doi.org/10.1016/j.biortech.2004.04.007>
- Hill, W. J., & Hunter, W. G. (1966). A Review of Response Surface Methodology: a literature Survey. *Technometrics*, 8(4), 571–590.
- Isaias, M., Núcleo, M., Planejamento, I. De, & Copersucar, C. C. T. C. (2004). *Assessment of greenhouse gas emissions in the production and use of fuel ethanol in Brazil*. SAO PAULO: Government of the State of São Paulo.
- Jones, R. P., Pamment, N., & Greenfield, P. F. (1981). *Alcohol fermentation by yeasts - the effect of environmental and other variables*.
- Koske, J. K., Kosgei, M. K., & Mutiso, J. M. (2011). *a New Third Order Rotatable Design in Five Dimensions*. 13(1), 157–163.
- Krakwowski, T., Polytechnic, F., & State, B. (1997). *Production of Ethyl Alcohol From Molasses Using Continuous Process*. 8(I), 35–39.
- Montgomery, D. C. (2005). *Design and Analysis of Experiments* (Fifth Edit). New York: John Wiley & sons.
- Razmovski, R., & Vucurovic, V. (2011). Enzyme and Microbial Technology Ethanol production from sugar beet molasses by *S. cerevisiae* entrapped in an alginate – maize stem ground tissue matrix. *Enzyme and Microbial Technology*, 48, 378–385. <https://doi.org/10.1016/j.enzmictec.2010.12.015>



- Tropea, A., Wilson, D., Torre, L. G. La, Curto, R. B. Lo, Saugman, P., Troy-Davies, P., ... Waldron, K. W. (2014). Bioethanol Production From Pineapple Wastes. *Journal of Food Research*, 3(4), 60. <https://doi.org/10.5539/jfr.v3n4p60>
- Tsuyoshi, N., Fudou, R., Yamanaka, S., & Kozaki, M. (2005). Identification of yeast strains isolated from marcha in Sikkim , a microbial starter for amylyolytic fermentation. *International Journal of Food Microbiology*, 99, 135–146. <https://doi.org/10.1016/j.ijfoodmicro.2004.08.011>
- Wyman, C. E., & Hinman, N. D. (1990). *Ethanol*. 24, 735–736.

**First A. Author (Kabue Timothy Gichuki)** Born in Nyeri in Central Kenya. Holds a Bachelors of Education Science Second Class Honours (Upper Division) in Double Mathematics in 1994 and a Master's Degree in Mathematical Statistics from Kenyatta University (2010) and currently a Phd student in Moi university in biostatistics.