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Optimization of Bioethanol Production from Sweet Sorghum Stalk (*Sorghum bicolor* (l.) *Moench*) Juice Using Response Surface Method

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Abstract: The use of fossil fuel as a source of energy has been unsustainable and has adverse effects to the environment. Bioethanol is a suitable alternative due to its exceptional properties. Bioethanol production can be done through fermentation of sucrose in presence of a catalyst and as is customary for every production processes, the fermentation parameters such as the pH, duration of reaction, the catalyst concentration and the temperature need to be optimized. Thus, this study sought to optimize bioethanol production parameters from the sweet sorghum stalk juice. Sweet sorghum is potential multipurpose crop since it can be used as human food, animal feed, animal fodder and processed for syrup and bio-fuel. For this work, Sweet sorghum stalks were harvested 15 weeks after planting, crushed to extract the juice and the juice fermented in presence of biocatalyst (*Saccharymyes ceresiae*). A 4^4 Factorial design in Minitab 17 software was used to design the experimental runs. Thereafter, response surface method (SRM) and contour plots were used to determine the best operating conditions among the applied factorial combination of parameters. It was concluded that the optimal catalyst concentration was 1.5 ± 0.5 g/l, duration of reaction was 55.25 ± 3.25 hrs., pH was 5.0 ± 0.25 and the temperature was 40 ± 1.0 degrees Celsius. The chemical composition of the produced bioethanol indicated that it is a good substitute for combustion engine fuel. Thus, the bioethanol has the potential to replace the fossil gasoline as a fuel hence being friendlier to the environment.

Keywords: Bioethanol, Response Surface Methodology (RSM), Fossil Fuel, Sweet Sorghum, Emissions

1. Introduction

The cost of fossil fuels and future of the oil supplies to developing world has been an emerging concern. Currently, fossil fuel use has been so unreliable and with adverse effects to the environment. These emerging concerns have prompted researchers to seek alternative sources of fuel for automotive transport. The substitute fuel will enable the affected countries to increase energy use, efficiency and reliability. In order to overcome the growing energy concerns, research on renewable energy sources has been of great interest with an effort to reduce the dependence on fossil fuels [1]. Specifically, bioethanol which is a cleaner-burning combustion engine fuel and considered environmentally safe as its green-house-gas (GHG) emission are less than those of fossil fuels. Pure bioethanol upon combustion emits carbon dioxide and water only (see equation 1) making it a carbon neutral source of energy [1]:

$$CH_3CH_2OH_{(l)} + 3O_{(g)} \longrightarrow 2CO_{2(g)} + 2H_2O_{(l)} \quad (1)$$

However, fossil fuels contain substantial amounts of additional elements such as nitrogen, sulfur and other hydrocarbons and once burnt, it emits several pollutants to the atmosphere such as Carbon monoxide, Carbon dioxide, Sulphur oxides, Nitrogen oxide and various hydrocarbon [2].

In the tropics, bioethanol is mainly sourced from plant such as sweet sorghum stalks juice, water hyacinth, corn, sugar cane, and cynic cassava among others. Production of first-generation bio-fuel from crops such corn and sugarcane sparked the food verses fuel debate increasing the emphasis on food security [3]. Thus, sweet sorghum (SS) plant which is grown mostly in the Tropics provide a potential feedstock that can be used for the second-generation biofuel production. Sweet sorghum has the following advantages as compared to sugarcane and corn ethanol which have been used previously; i) high sugar content in its stalk and ii) can be directly fermented, iii) requires minimum water and fertilizer requirements for growth, iv) drought and more salt resistance with adaptability to extreme environmental temperatures and v) the harvesting period is short [4]. Consequently, sweet sorghum has competitive potential ethanol yield as compared to other second-generation feedstock such as wheat and rice straw [5].

Mei *et al*, [6] asserts that sweet sorghum juice could be directly fermented in presence of a catalyst (yeast) and converted to bioethanol. In this study, the catalyst that was used is *Saccharymyes ceresiae*. Fermentation reaction processes and ethanol yields are usually affected by temperature, pH, duration of reaction and yeast concentration among other factors. However, the literature reports on bioethanol production optimal production parameters as: a pH of 5.0-7.0, duration of reaction of around 36-72 hours, temperature of 30-45°C and catalyst concentration of 1-4 g/l [7]. Such production conditions are always reported as a range which is a challenge to implement during industrial production. There was therefore a need to establish specific conditions for production of bioethanol for the sweet sorghum juice fermentation process in this study.

In practice, there are several methods of optimization using Minitab-17 software such as response surface, factorial design, and Taguchi method among others. In this study, factorial design was used on its strength to predict self-effects as well as the interactions between different variables involved in the experiment and ease of graphical analysis by surface response surface - contour plots [8]. Thereafter, response surface and contour plots were applied for optimizing fermentation parameters.

To obtain bioethanol thereafter, the fermentation product was then distilled and characterized to establish its suitability as an ideal fuel for combustion engines in terms of chemical composition. According to Ağbulut, Ü. et al. [1], the use of sweet sorghum juice as combustion engine fuel have been embraced despite the chemical composition of the co-fermentation products from sweet sorghum stalk juice. As the fermentation process rarely produce a single product depending on the chemical composition of the stalk juice, the exact chemical co-products of the produced bioethanol was also investigated in this study. The nature of chemical composition of the bioethanol will make it possible to predict the combustion engine emission products as shown in equation 1.

The use of ethanol eliminates the noxious emissions from the combustion engines of oxides of nitrogen and sulphur and particulate emissions and can be used in automotive engine mainly as additive for diesel or gasoline [9]. Thus ethanol keeps the engine clean, it burns completely and at a slightly cooler temperatures compared to gasoline. It burns well because it is oxygenated and the extra oxygen molecules in ethanol aids it to burn better.

2. Materials and Methods

2.1. Chemical and Reagents

Sweet sorghum variety, IESV 92001 DL, seed was sourced from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi Office-Kenya, Yeast (*Saccharymyes ceresiae*) was obtained commercially from Muhoroni agrochemicals and food industries. The NaOH and H_2SO_4 of analar grades were obtained from Kobian chemical company, Nairobi (Kenya).

2.2. Sweet Sorghum Stalk Juice

The Sweet sorghum variety, IESV 92001 DL was identified from six other varieties under agronomic evaluation trials at Maseno University as having the highest brix content. The Sweet sorghum varieties were grown at Maseno University experimental farm located at 0° 0'23.64'S latitude and 34° 35'48.9' E longitude. The altitude of Maseno is 1,503 metres or 4,934 feet above sea level with a mean annual daily temperature of 20.6°C and the soils are classified as with a pH of 5.4. The mean annual rainfall 1820 mm, with the short rains having a mean rainfall amount of 79.30 mm [10]. The sweet sorghum was planted during the short rainy season of August - December 2022 at Maseno experimental farm. Harvesting was done manually in triplicates at the 15th week, where the sweet sorghum plants were selected randomly, their leaves, heads, and pinnacles stripped and °Brix determined. A parallel, unreported, work on the same variety determined that at 15th week, the brix level was maximum. The juice was extracted using electrical stalk juice crushers and filtered.

Total Sugars in terms of °Brix was measured using a digital refractometer (Model PAL1, Atago Co. Ltd., Tokyo, Japan).

2.3. Optimization of the Bioethanol Production Parameters

Fermentation process was optimized using different combinations of the fermentation conditions. Table 1 shows the four factors and four levels for each factor which were considered in the bioethanol optimization process.

Table 1. *Levels of process parameters used in experimental design.*

Factors	Levels			
Temperature (°C)	30	35	40	45
pH (a. u)	4	5	6	7
Catalyst conc. (g/l)	0.5	1.5	2.5	3.5
Time (hrs)	40	55	70	85

The factorial design experiment was developed with the help of Minitab 17 software with a total of 256 combinations (See Table 5). The experimental combinations were performed and the resulting response was analyzed statistically using Minitab 17 software to obtain the optimal conditions. The best combination was then used to produce a larger amount of bioethanol. The percentage yield of bioethanol was calculated using equation 2.

2.4. Fermentation of Juice to Bioethanol

Bioethanol was obtained from the sweet sorghum juice in anaerobic batch fermentation process. 60 ml of juice was put in a 250 ml conical flask, and the pH of the solution adjusted accordingly using dilute solutions of either NaOH or H_2SO_4. The adjusted solutions were placed in a water bath, preheated at specific temperature (30, 35, 40, 45), for 60 minutes. After temperature stabilization the required amount (0.5 g, 1.5 g, 2.5 g, 3.5g) of yeast was added to the conical flask and the broth was left to ferment for predetermined durations of time (Table 1). After fermentation, the quantity of ethanol obtained was determined from gas chromatography. The percentage bioethanol yield per gram of juice from the fermentation process was calculated using equation (2) [11];

$$\text{Bioethanol yield/g (\%)} = \frac{Weight\ of\ bioethanol\ obtained\ (g)}{Weight\ of\ juice\ used\ (g)} \times 100\% \quad (2).$$

2.5. Chemical Composition of Fermentation Product

It is worthy to note that analysis was done just after the initial distillations so as not to lose other components of the fermentation. Bioethanol concentration was determined by a gas chromatography, GC-9A Shimadzu fitted with the flame ionization detector and equipped with a packed column of size 30 m by 0.25 mm by 0.25 µm and a flame ionization detector. The temperature of the injector was 200°C and that of the detector 250°C. For the column, a gradient of 50-170°C was used and nitrogen was used as a carrier gas while hydrogen (40 ml/min) and air were used as the combustion gases at a flow of 200 ml/min. Samples of 1 µl were directly injected into the column in replicates at a split ratio of 10:1 and the concentrations of different alcohols contents were determined from peak areas of the obtained chromatograms [11].

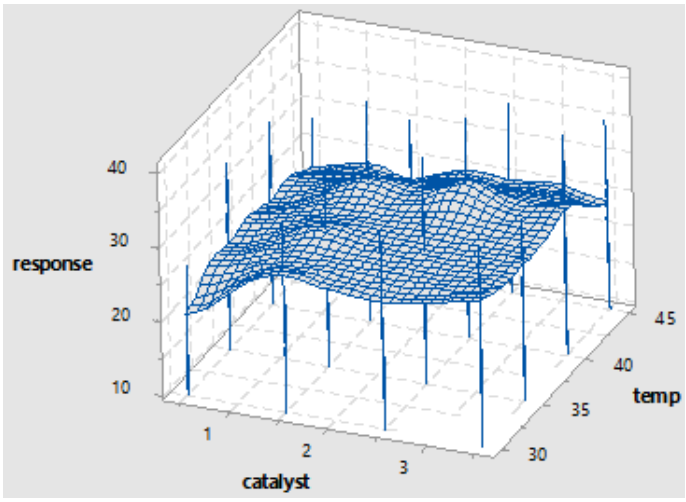
3. Results and Discussion

3.1. Optimal Conditions for Bioethanol Production

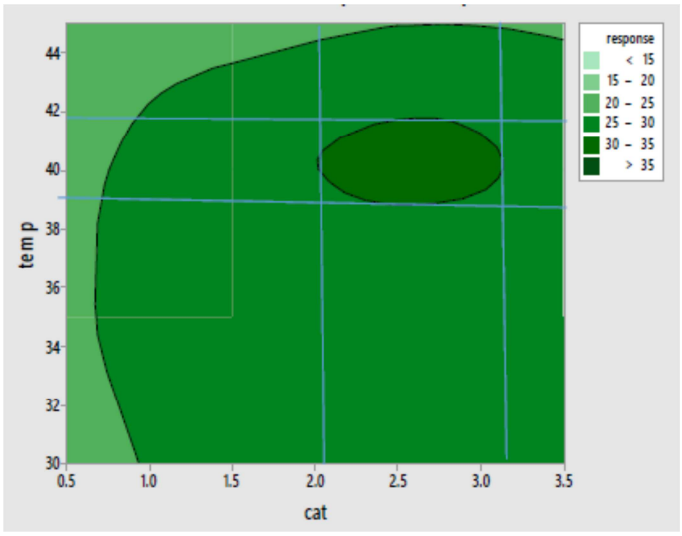
The objective of this study was to determine the optimal laboratory fermentation process conditions necessary to achieve high ethanol yields. Considered conditions included temperature, pH, reaction time and catalyst concentration which have influence on ethanol productivity [12]. For the combinations, the 4^4 (256 combinations) generated runs were as per Table 5. Minitab 17 was then applied to develop the response surface plot presented in Figure 1a and in appendices 2 to 5. The 3-D surface plots and the contour plots (Figure 1b) showed a relationship between two variables at a time while maintained third variable at a constant level. For example; Figure 1 is for the relationship between catalyst concentration and temperature when the pH and temperature were fixed at 5 and 70), respectively. In this germane attempt, the response surface of a 3-D plots is used to explain how two processes parameters interacted with each other when the third process parameter was fixed at given level and represented in the accompanying 2-D plot. The response surface was then used to display the contours for any selected two parameters at a time and to a pre-selected degree of accuracy (in this case to 1 decimal places as revealed by the scale used in the graph). For surface response method (RSM), the contours are used to represent the response peak parameters for the maximum response. For example, in Figure 1, each colored levels in the 2-D contour represents the response of the ethanol yield. The region of maximum response value (labelled R) of ethanol yield was located on the area confined in the smallest ellipse indicated by the darker color in the contour plot (Figure 1b). From Figure 1, it was depicted that at lower catalyst loading, the general response is a bit lower. At higher catalysts loading, the general trend is higher responses at some temperature values. Generally, the response is varied; being higher at some intermediate catalyst amounts and also varied at some temperature amounts. To get a better perspective of the type of surface, a 2-D contour plot was made to highlight the area of optimum values of the catalyst load and the accompanying temperature. Thus a germane attempt is made, in this work, in obtaining the optimum parameters ranges responsible for the highest points as the area enclosed between the grid lines in Figure 1. The contour plot presents an easier, less mathematical, way of determining the level of response to consider as optimum.

The 2-D contour plot accorded us the opportunity to determine the required values. From grids in Figure 1, the optimum catalyst ratio is between 2.00 – 3.25, while the optimum temperature is seen to be 39 – 41°C. Similar considerations in obtaining optimum parameters were then performed for all combinations of the four parameters of pH, catalysts concentration, temperature, and reaction time and the outcomes were recorded in Table 2 (see Figures 2-4 for additional information). To obtain the overall optimum condition for each parameter, the pre-determined ranges of the four parameters were determined by inclusivity areas (see table 2) where only the common range (second last row) was generated and the average (last row) becomes the optimal condition. For example, consider the column for temperature with ranges such as 39-41, 36.5 -42.5, and 38-42. Meaning the lowest common inclusive point is 39 while the highest inclusive common point is 41. Thus the average obtained range is 39 - 41 with a midpoint of 40 and the variance of ±1.0. Thus the optimum Temperature was 40 ± 1.0.

Therefore, the optimal conditions achieved were as follows: temperature at 40 ±1.0°C, catalyst concentration of 1.5 ± 0.5 g/l a pH of 5.0 ± 0.25 and reaction time at 55.25 ± 3.25 hours.



(a)



(b)

Figure 1. *a) 3-D Surface plot and b) contour plot for Catalyst conc./substrate ratio, Temperature combination verses the response (where the pH = 5 and Temperature 70).*

Table 2. *The Optimum condition for the fermentation process.*

Combinations	Optimum conditions			
	Temp (°C)	Cat. Conc/substrate ratio	pH (a. u)	Time (hrs.)
Cat Vs Temp	39–41	2.00-3.25	n.a	n.a
Cat. Vs pH	n.a	1.2-2.3	5.0-6.1	n.a
Cat. Vs Time	n.a	0.5–3.5	n.a	40–85
pH Vs Temp	36.5-42.5	n.a	4.6-7.0	n.a
pH Vs Time	n.a	n.a	5.25–5.75	52–59
Temp. Vs Time	38–42	n.a	n.a	65-82.5
Common range	39-41	1.2-2.3	5.25-5.75	52-59
Average	40±1.0	1.5±0.5	5.0±0.25	55.25 ± 3.25

Key: a.u = arbitrary units, n.a = not applicable.

Table 3 show the obtained optimum values in comparison with the literature reports. From literature, the catalyst concentration was similar to those reported by Makori, [13] and Wang, L, et al, [8]. The pH was also similar to those reported by Mutepe, [15]. The duration of reaction only recorded values in close range but not similar to those reported by Makori, [13]. However, the temperature ranges of 40 ± 1.0°C were higher compared to what is in the

literature (see Table 3). It is reported that higher temperature generally causes more collisions among the molecules and therefore increases the rate of a reaction. More collisions increase the likelihood that substrate will collide with the active site of the enzyme, thus increasing the rate of an enzyme-catalyzed reaction. Above the optimal temperature, the reaction begins to decline because the enzyme becomes denatured. The rate of chemical reactions therefore increases with temperature but then decreases as enzymes denature [14].

Table 3. *Optimum fermentation condition for Sweet sorghum juice.*

S. No	Duration of reaction (hrs.)	pH	Temp (°C)	Catalyst concentration (g/l)	Remarks (Yeast used)	Ref
1	55.25±3.25	5.0±0.25	40±1.0	1.5±0.5	*Saccharomyces Cerevisiae*	This work
2	72	4.5	30	2	*Saccharomyces Cerevisiae*	(13)
3	60	5.0	33	2	*Zymomonas Mobilis*	(8)
4	72	5	35	1	*Saccharomyces Cerevisiae*	(7)
5	65	4.5	30	3	*Saccharomyces Cerevisiae*	(16)

The observed differences especially the temperature could be due to different conditions and the method used in the fermentation process or rather the process of optimization. This study obtained a concentration of 1.5±0.5 g/l while Makori, [13] and Mutepe [15], got a concentration of 2 g/l. Catalyst are substances that lowers the activation energy of the reaction positioning the molecules and accelerating the speed of reaction. Here, the literature values and the data obtained for this work are statistically similar. Once more the reason could be due to the similarity of the enzyme used. The procedure was done to know the exact range of catalyst that would be used in production of bioethanol. Beyond this range, the yeast does not convert the excess quantity of sugar present and as a result the excess sugar goes to waste.

Specifically, this study obtained an optimum pH of 5.0 ± 0.25, Marrow [14] got a pH of 5.0 while Mukabane, [11] got a pH of 4.5. These study findings and those of Mukabane, [11] were similar unlike those of Makori,[13] that were similar only at 2d. This could be due to the time of reaction or the yeast type used or the juice used. The similarities indicate that the system used in this study is not very different from that reported in literature. However, unlike the other literature information, for the first time an exact suitable pH range for fermentation was experimentally established. The pH is a strong factor that interferes with the fermentative processes rate. Moreover, lower pH in fermentative medium inhibits the yeasts cell growth and nutrition material exchange between the cells because a lot of H^+ surrounding the enzyme and the surface of the substrate will be positively charged [16]. Hence, the substrate and the enzyme cannot bind together because the charges would be repelling while higher pH will enhance denaturing of the microbial. Both lower and higher pH's lower the bioethanol yield consequently. Therefore, based on the study, the suitable pH for the bioethanol production at a higher yield would be 5.0.

The optimum reaction duration of 55.25±3.25 hrs. was established for this study. This value was distinct from the recorded work in the literature. The variation could be due to the amount of sugar in the substrate used in the study not forgetting to mention the degrees of temperature used. The higher the temperature, the faster the reaction, since temperature increases the kinetic energy of the molecules present in the broth which gives room for more reaction. Moreover, enzyme reaction is reversible, during the initial stages of the reaction, there is no product present in the broth and therefore, the reaction proceeds to the forward reaction. However, as the reaction continues there is a significant accumulation of the product and hence the back reaction is observed, as a result, the formation of the product slows down the process thus the right duration should be known because if the incubation time is too long the measured activity of the enzyme might be falsely low [13].

This study obtained 40 ± 1.0°C as optimum temperature for the reaction, while Mukabane, [11] got temperature of 33°C while Makori [13], reported a temperature of 30°C. The literature values are statistically different from the value obtained from this study. One possible cause of this disparity could be enzymatic activity involved in ethanol production where temperature exerts a profound effect on all aspects of yeast growth, metabolism and fermentation [17]. Increasing temperature increases the kinetic energy of molecules and the amount of the activation energy which speed the rate of reaction thus binding of the substrate and the enzyme. Either, too high temperature would denature the enzymes involved in the medium and thus inactivating the enzymes while lower temperature consequently inactivates the enzymes which, consequently, lowers the ability to catalyze the intended reaction [12].

3.2. Characterization of the Fermentation Product

Gas chromatography was used to identify the chemical composition of the fermentation products. The results of molecular composition their respective concentrations and the chemical formulae are in Table 4.

Table 4. *The chemical composition, concentrations and chemical formulae of the fermentation products.*

Characteristic	Concentration		Chemical formula
	Quantity	Unit	
Acetaldehyde	2.38	Ppm	CH_3CHO
Ethyl acetate	5.92	Ppm	$CH_3COOC_2H_5$
Methanol	6.42	Ppm	CH_3OH
n-propanol	26.61	Ppm	C_3H_7OH
2-methylpropanol	72.51	Ppm	C_4H_9OH
Acetic acid	34.57	Ppm	CH_3COOH
3-methybutanol	125.66	Ppm	$C_5H_{11}OH$
Propionic acid	8.75	Ppm	C_2H_5COOH
Butyric acid	7.44	ppm	C_3H_7COOH
Ethanol	8.86%	V/V	C_2H_5OH

V/V= Volume/Volume

It is noted that the major component is ethanol at 8.86 %. It is noteworthy that available literature only reports the purified levels of bioethanol products. The strength of the bioethanol was, however, low and this was due to the sample having been only purified by a single simple distillation process. The simple distillation was deliberate so that most of the possible contaminants of bioethanol, as a fuel, are quantified. It was necessary to get all the components so that once the bioethanol is used in combustion engines, all possible combustion products can be predicted.

From their chemical formulae in the last column of Table 4 it is noted that Sulphur and nitrogen containing compounds are missing. The absence of the N and S containing compounds are in support of the analysis in Table 4 that indicated that the Sulphur content was within the ASTM allowed limits. This, therefore means that the bio-fuel produced may not emit Sulphur compounds when used as a combustion engine fuel.

4. Conclusion

Combination of the factorial experimental design, the response surface 3-D plots and the contour plot was an effective method of optimizing the fermentation parameters such as the catalyst concentration, duration of the reaction, pH and temperature such that the optimal catalyst concentration was 1.5±0.5 g/l, duration of reaction was 55.25±3.25 hrs., pH was 5.0±0.25 and the temperature was 40±1.0°C. In addition, the analysis of the bioethanol using the Gas chromatography ascertained that the major product formed was ethanol. However, during fermentation there are by-product formed depended on the level of chemical composition of the extracted stalk juice but the level of compounds such as Sulphur and nitrogen with adverse effects to the environment were so minimum (see table 4). Therefore, the bioethanol produced once burnt may not emit harmful nitrogen oxides or Sulphur oxides to the environment.

5. Recommendations

For maximum bioethanol productivity and yield and minimal chances of side-products, the following optimal fermentation conditions: pH of 5.0±0.25, temperature of 40±1.0 ^{o}C, reaction time as 55.25 ± 3.25 hours and catalyst conc./substrate ratio of 1.5±0.5 g/l are recommended. The above optimum conditions resulted into products that did not result into any environmentally harmful combustion product. Finally, the produced ethanol from sweet sorghum stalk juice was within the required quality needed for automobile transport and it's recommended as a suitable alternative for the gasoline used currently.

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Appendix

Table 5. *The statistical combination of the optimum conditions.*

	Temperature	pH	catalyst	Time
1	30	5	3.5	55
2	40	5	3.5	40
3	35	5	2.5	40
4	40	7	2.5	70
5	35	6	1.5	85
6	40	7	0.5	55
7	35	7	3.5	85
8	40	5	3.5	85
9	40	4	3.5	55
10	45	7	2.5	40
11	45	6	0.5	55
12	30	4	0.5	85
13	30	6	3.5	40
14	35	4	2.5	70
15	35	5	3.5	40
16	30	5	0.5	70
17	35	7	0.5	55
18	40	4	2.5	85
19	45	4	1.5	70
20	45	5	1.5	70
21	40	6	0.5	70
22	30	5	3.5	70
23	30	6	2.5	55
24	40	4	0.5	70
25	30	5	0.5	40
26	30	4	1.5	85
27	45	7	2.5	55
28	35	6	0.5	55
29	40	4	2.5	70
30	30	4	2.5	70
31	30	7	3.5	55
32	35	5	1.5	85
33	40	6	2.5	40
34	45	6	2.5	70
35	40	7	3.5	40
36	30	7	2.5	55
37	35	5	1.5	40
38	30	5	2.5	70
39	30	6	0.5	55
40	35	4	1.5	40
41	35	6	0.5	85
42	35	6	3.5	40
43	35	7	3.5	55
44	45	7	3.5	40
45	30	5	3.5	85
46	35	5	1.5	70
47	35	5	3.5	70
48	35	6	3.5	55
49	35	6	0.5	70
50	30	7	1.5	40
51	40	4	2.5	40
52	35	7	2.5	85
53	30	6	0.5	70
54	45	4	2.5	55
55	40	7	2.5	55
56	30	6	1.5	70
57	40	5	1.5	40
58	35	5	0.5	40
59	45	5	2.5	70

	Temperature	pH	catalyst	Time
60	35	7	0.5	40
61	40	6	0.5	55
62	40	7	1.5	85
63	45	6	1.5	40
64	35	7	2.5	85
65	30	7	2.5	40
66	45	5	3.5	85
67	30	5	0.5	85
68	40	6	2.5	85
69	35	7	0.5	85
70	35	4	3.5	85
71	40	6	3.5	85
72	30	6	0.5	85
73	35	7	1.5	55
74	35	7	3.5	40
75	40	4	0.5	85
76	40	4	1.5	70
77	35	5	0.5	55
78	30	7	3.5	85
79	45	4	3.5	85
80	40	7	3.5	70
81	35	6	2.5	55
82	40	6	0.5	40
83	45	4	2.5	85
84	40	5	0.5	70
85	40	7	0.5	40
86	40	5	0.5	55
87	30	7	1.5	70
88	40	7	2.5	40
89	30	5	0.5	55
90	45	7	3.5	70
91	45	6	0.5	85
92	30	4	3.5	40
93	45	4	0.5	55
94	45	7	2.5	70
95	40	6	3.5	55
96	45	5	3.5	40
97	45	5	2.5	55
98	30	7	0.5	40
99	35	4	0.5	55
100	40	5	2.5	70
101	40	4	1.5	40
102	30	6	1.5	85
103	35	5	0.5	70
104	30	6	2.5	85
105	40	5	0.5	85
106	30	4	2.5	85
107	40	6	0.5	85
108	35	7	2.5	70
109	40	6	3.5	70
110	40	5	3.5	55
111	35	4	0.5	70
112	35	4	1.5	70
113	30	5	1.5	55
114	45	5	1.5	70
115	40	5	2.5	55
116	35	4	0.5	40
117	30	4	2.5	40
118	35	4	2.5	55
119	40	7	2.5	85
120	30	4	0.5	55
121	45	5	1.5	85
122	35	7	1.5	70
123	40	4	2.5	70
124	35	6	3.5	70
125	30	6	3.5	70

	Temperature	pH	catalyst	Time
126	45	4	1.5	40
127	45	6	1.5	85
128	45	6	0.5	70
129	45	5	0.5	55
130	30	5	1.5	85
131	45	4	0.5	70
132	45	7	3.5	55
133	30	5	2.5	85
134	30	4	1.5	55
135	45	5	0.5	40
136	35	4	3.5	55
137	40	7	3.5	55
138	40	6	1.5	40
139	40	6	1.5	70
140	35	5	2.5	85
141	35	6	1.5	55
142	45	5	1.5	40
143	40	6	3.5	40
144	45	7	0.5	70
145	30	4	3.5	85
146	40	5	1.5	85
147	45	7	2.5	85
148	30	7	0.5	70
149	45	6	2.5	85
150	35	6	0.5	40
151	30	7	1.5	70
152	35	4	3.5	70
153	35	5	3.5	85
154	45	5	1.5	55
155	30	4	0.5	70
156	35	4	1.5	55
157	45	7	2.5	40
158	45	7	2.5	85
159	40	5	1.5	55
160	30	7	0.5	55
161	35	7	2.5	40
162	40	7	0.5	70
163	30	7	3.5	40
164	30	7	2.5	70
165	40	4	3.5	40
166	45	7	0.5	55
167	45	7	3.5	85
168	40	4	0.5	40
169	40	4	3.5	85
170	45	6	3.5	55
171	45	4	0.5	85
172	30	6	3.5	85
173	45	4	2.5	85
174	30	6	1.5	40
175	35	6	2.5	40
176	45	6	3.5	85
177	40	6	2.5	70
178	35	4	3.5	70
179	30	7	1.5	85
180	30	7	1.5	55
181	30	5	2.5	40
182	40	6	1.5	70
183	40	7	3.5	85
184	35	7	1.5	40
185	40	7	0.5	85
186	45	6	0.5	40
187	45	6	3.5	70
188	45	6	1.5	55
189	30	5	1.5	40
190	40	7	1.5	40
191	30	4	0.5	40

	Temperature	pH	catalyst	Time
192	35	6	3.5	85
193	30	6	1.5	55
194	35	5	1.5	55
195	35	7	2.5	55
196	35	4	1.5	85
197	40	4	0.5	55
198	35	5	0.5	85
199	35	6	1.5	40
200	45	4	3.5	40
201	35	7	0.5	70
202	45	7	1.5	55
203	35	5	2.5	55
204	30	4	3.5	70
205	45	4	2.5	40
206	35	4	0.5	85
207	30	4	2.5	55
208	40	4	3.5	70
209	30	4	1.5	70
210	45	4	2.5	70
211	45	6	3.5	40
212	30	6	2.5	70
213	40	7	1.5	55
214	40	5	1.5	70
215	30	5	2.5	55
216	35	6	2.5	85
217	30	5	3.5	40
218	40	5	0.5	40
219	45	5	2.5	40
220	45	4	3.5	70
221	30	6	2.5	40
222	35	4	2.5	40
223	40	4	1.5	85
224	35	4	3.5	40
225	45	4	3.5	55
226	45	7	0.5	85
227	45	4	1.5	55
228	45	5	3.5	70
229	35	5	3.5	55
230	40	5	3.5	70
231	30	4	2.5	40
232	45	6	2.5	55
233	40	4	1.5	55
234	45	7	0.5	40
235	40	6	1.5	55
236	35	7	1.5	70
237	45	5	2.5	85
238	30	7	3.5	70
239	40	6	1.5	85
240	35	4	2.5	85
241	45	5	0.5	85
242	40	6	2.5	55
243	35	6	2.5	70
244	40	4	2.5	55
245	45	5	3.5	55
246	30	4	3.5	55
247	30	7	2.5	85
248	40	5	2.5	85
249	35	5	2.5	70
250	30	6	3.5	55
251	30	7	0.5	85
252	40	5	2.5	40
253	45	4	0.5	40
254	30	6	0.5	40
255	45	6	2.5	40
256	45	7	1.5	70

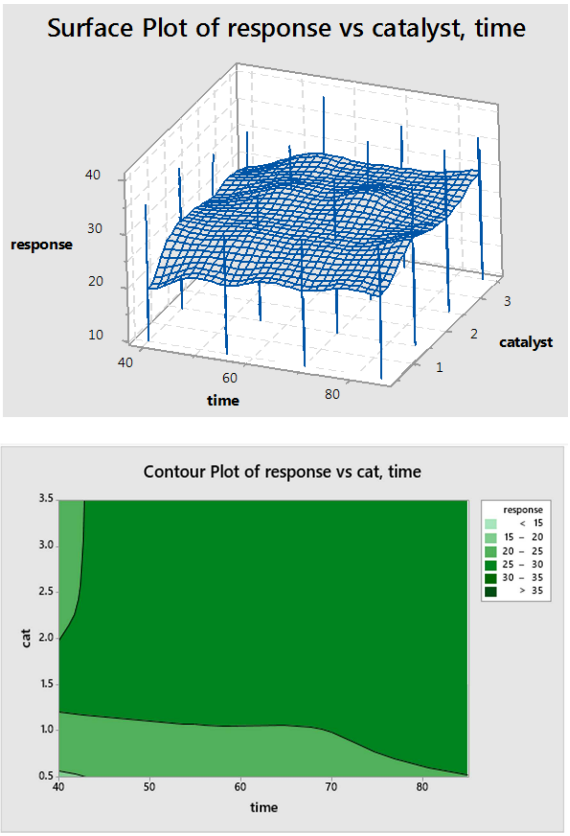

Figure 2. *The Surface plot and contour plot for Catalyst, reaction time combination verses the response.*

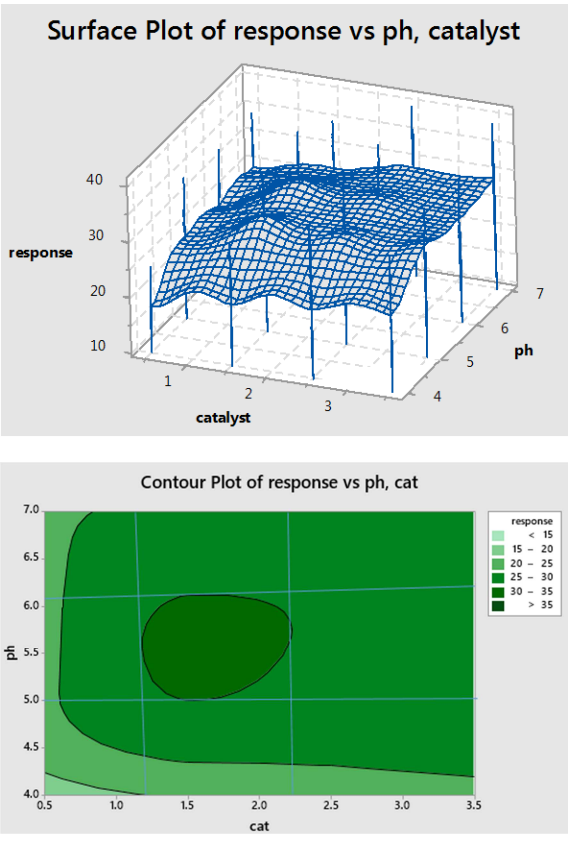

Figure 3. *The Surface plot and contour plot for Catalyst, pH combination verses the response.*

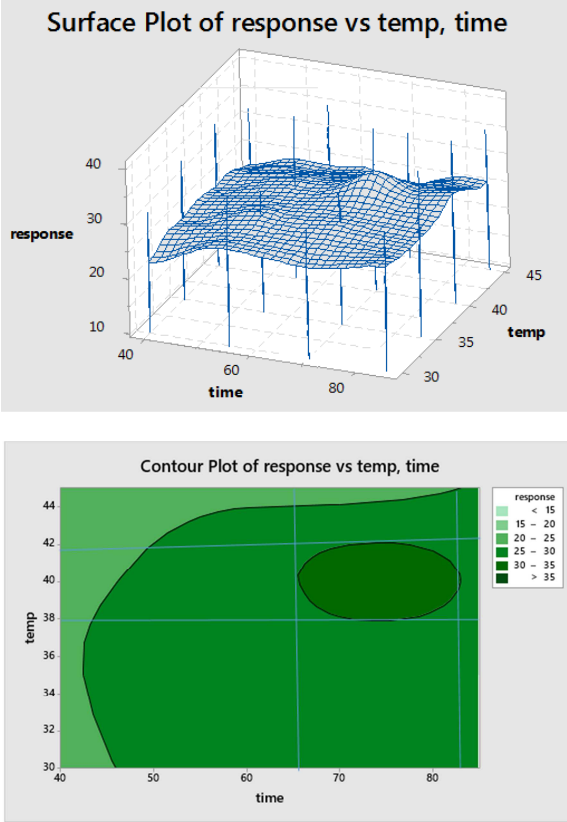

Figure 4. *The Surface plot and contour plot for temperature, reaction time combination verses the response.*

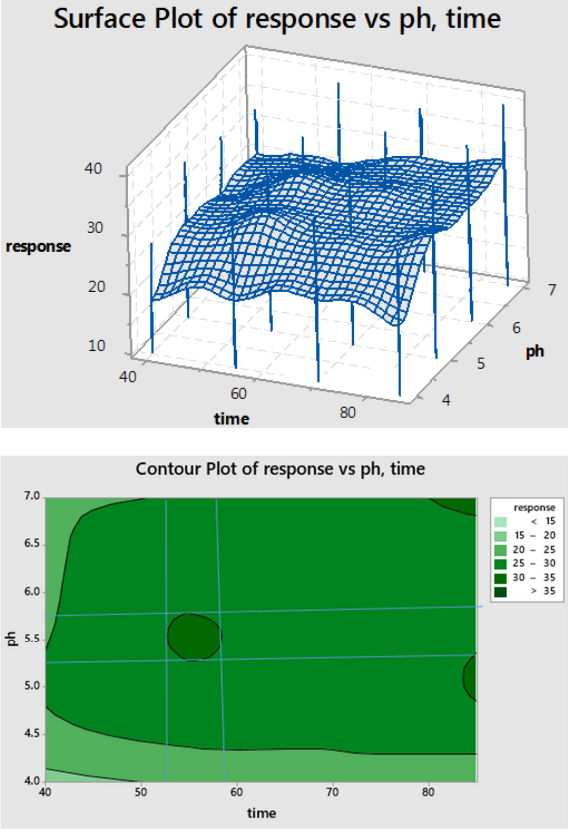

Figure 5. *The Surface plot and contour plot for pH, reaction time combination verses the response.*

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