

Optimization of Brewing Extract for Optimum Yield of Beer from Industrial Beet Molasses

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Abstract- One of the critical challenges faced by Breweries in Nigeria and other brewing Industries in Africa is inability to meet the gross line yield (GLY) target. During the mashing phase, not all extract is filtered out of grist and as such, some percentage of the extract is discharged as spent grains. This contributes to the shortage of wort which contains the sugar molecules that would undergo fermentation to produce ethanol. Outside technical and logistics problems, the rapid increase in raw material costs and wages makes it more than ever essential to optimize raw materials. This study is aimed at assessing the production level and extract loss in a brewing company which could be used to appraise the productivity of brewing companies in Nigeria and Africa at large. The study was done through certain formation of concentration of the constituents of production data, which were given as: sugar concentration (g/l) = 325 (max.), yeast concentration (g/l) = 1 (max.) and nutrient concentration(g/l) = 4 (max.). Using the design expert software to develop the orthogonal array of the mixture design process. Chemical analyses of the production data mix were conducted after the mixture were subjected to the standard reaction conditions to observe the extent of the ethanol yield per mixture size. Then the total data array were obtained and ready for statistical analyses with software for the test of significant results. The result of the statistical analysis showed that a total average value of ethanol yield of - 1436800475.25 g/l/d was obtained. The model that generated this value accounted to 99.99 percentage with signal/noise to be 308.102, implied adequate signal.

Index Terms - Optimization, Brewing Extract, Ethanol yield, Beet Molasses.

I. INTRODUCTION

The value chain of any industry is a valuable tool to evaluate whether and how the competitive advantage is achieved, maintained and defended as well as how the economy is sustained. For the brewing sector, many industries depend on the sector for sustenance. From production to consumption, the brewery industry has a long supply chain and accounts for a larger contribution to the GDP than what is presently captured. According to Statista, revenue in the Nigeria beer market is projected to reach US\$3.31m in 2024. The report also stated that the market is expected to show an annual growth rate (CAGR 2024-2029) of 3.855, resulting in a projected market volume of US\$4.00m by 2029 (Jan, 2024).

Among the ten largest countries in the world, Nigeria is growing the most rapidly. Consequently, the population of Nigeria, currently the world's 7th largest, is projected to surpass that of the United States and become the third largest country in the world shortly before 2050, according to the U.N., and the continent as a whole will boast the largest working (and drinking) age demographic by 2050. Growth in beer consumption is expected to continue at 5 percent year-over-year from 2015 to 2025, outpacing Asia, the Middle East and North Africa (all pegged at 3 percent) and far ahead of crickety old markets like Western Europe and North America (pegged at 1 percent or less). This growth demands a complimentary amount of research and understanding. This research work attempts to

encapsulate the various complexities that go into the brewing process by breaking it down to a more understandable level.

One of the critical challenges faced by Breweries in Nigeria and other brewing Industries in Africa is inability to meet the gross line yield (GLY) target. During the mashing phase, not all extract is filtered out of grist and as such, some percentage of the extract is discharged as spent grains. This contributes to the shortage of wort which contains the sugar molecules that would undergo fermentation to produce ethanol. Aside technical and logistics problems, the rapid increase in raw material costs and wages makes it more than ever essential to optimize raw materials. The beer supply chain is a system of crucial steps taken from recipe to conception through production, brewing, bottling, and delivery to consumers. This includes sourcing raw materials such as barley, hops, and yeast. These raw materials are most times in scarcity due to their inherent seasonal variation, adverse weather condition, high cost of transportation, and other environmental event. With rising temperature reducing soil moisture and droughts exacerbating water shortages, climate change poses a real threat to the availability of raw materials of the beer supply chain. According to Vanguard (2024), the brewery industries are now facing severe cost pressure as prices of local raw materials rise astronomically undermining their backward integration strategy. The cost pressures coming from barley, sorghum, wheat and others would remain elevated, driven by the impact of rising inflation, insecurity across agricultural belts in Nigeria as well as other macroeconomic challenges. The objectives of this research work are: to optimize brewing raw materials for efficient reduction of extract loss and production of ethanol using a Central Composite Design – Response Surface Methodology; to develop a new method of operation module for extract loss reduction in brewing industries and to develop a mathematical model that expresses the relationship between the production yield and the independent factor variables. An optimization technique is vital in order to improve performance of the systems and increase the yield of the processes. This research work will serve to vindicate production engineers of different sectors and brewers to acknowledge the fact that the viability

of yeast as well as temperature and pressure in the tank has great impact on the quality and quantity of green beer produced during fermentation.

II. LITERATURE

The fermentation of malted barley during the brewing process generates ethanol Rajendram and Preedy 2009. Although non-alcoholic beers exist, the ethanol content of beer usually varies between 3% and 9% alcohol by volume, Rajendram and Preedy 2009. During the brewing process, fermentation of the starchsugars in the wort produces ethanol and carbonation in the resulting beer Barth, 2013. Beer is one of the oldest McFarland, 2009 and most widely consumed alcoholic drinks in the world European Beer Guide, 2006, and the third most popular drink overall after water and tea Max Nelson, 2005. Due to the high demand for beer in Africa, many Scholars have carried studies on beer production and have discussed some of the challenges faced by brewers which called for optimization of brewing raw materials and process to enhance low cost use of industrial sugar beet molasses for the efficient production of ethanol by yeast fermentation.

Sugar beet and cane molasses are abundant liquid by-products from the sugar industry, which are generally found at high amount of total sugars (50.6–71.0% w/w) and traces of micro nutrients such as minerals (Ca, Mg, Na and K), phosphate and nitrogen compounds (Palmonari et al., 2020). These sugar-rich solution does not require any major physical or chemical pretreatment (such as hydrolysis, filtration, sterilization, etc.) before fermentation, making them very appropriate for ethanol production. In this context, several studies already reported the efficient production of bio ethanol using sugar beet and sugarcane molasses. For instance, Razmovski et al. compared the fermentation performances of immobilized and free yeast cells using sugar beet molasses and thick juice at three initial glucose concentrations of 100, 200 and 300 g/L (Razmovski et al., 2012). For both substrates, immobilized yeasts resulted in higher ethanol yields than free cells condition, with the highest ethanol volumetric productivity of 1.257 and 1.422 g/L/h obtained with molasses and thick juice diluted at 150 g/L of initial sugar, respectively. In another study, the continuous

production of ethanol was investigated using an immobilized yeast cell reactor and sugarcane molasses as low-cost fermentation substrate (Ghorbani et al., 2011). The highest ethanol production of 19.15 g/L was obtained with a hydraulic retention time of 15.63 h combined with an initial sugar concentration of 150 g/L. In a recent study, a rotary biofilm reactor was developed for long-term bioethanol production using non-sterilised sugar beet molasses (Roukas et al., 2020). By recycling 30% of the fermentation broth every 36 h, a stable production of 52.3 g/L of ethanol was achieved over a period of 60 days. In addition, molasses can also be used for the production of different alcoholic beverages such as rum, a spirit distillate with an ethanol content of 37–43% alcohol by volume (Mangwanda et al. 2020). In another study, the ethanol production performances of corn mash feedstock were improved by the addition of sugarcane molasses (Alcantara et al. 2020). Mixing 50% of corn mash with 50% of sugar cane molasses generated the highest ethanol concentration (8.2%).

To implement an efficient fermentation process using industrial by-products, several process parameters such as sugar and yeast concentrations must be optimized to ensure high ethanol productivity while keeping in mind the economic aspects of the process (Darvishi et al., 2019). Nutrient supplementation is also an important parameter to take into consideration since an adequate amount of nutrient can significantly improve yeast viability and resistance to the medium, stimulating ethanol production performances. Since alcoholic fermentation is a complex biological process involving various operating factors, the use of the classical “one factor at a time” approach could be time-consuming due to the large number of experiments to perform. Hence, tools such as the statistical design of experiment (DoE) allow investigating the effect of several operational factors as well as their interactions on the overall process while considerably reducing the number of experimental tests (Keskin et al., 2016). For instance, a central composite design coupled with response surface methodology (CCDRSM) represents a powerful and effective statistical tool that could commonly be used for the optimization of biotechnological processes such as fermentation (Darvishi et al., 2019; Boboescu et al., 2017, and

Pattanakittivorakul et al., 2019). Once developed, the CCD-RSM can be used to predict a process output, while imposing specific constraints based on economical and technical aspects.

In this context, the present work aims to optimize the production of ethanol from non-treated sugar beet molasses produced by a local sugar refinery. For this purpose, a CCD-RSM was designed and developed to investigate the effect of three fermentation process parameters (initial sugar, yeast and nutrient concentrations) on ethanol productivity while considering several operating parameters such as ethanol yield and sugar utilization rate. Then, the second-order mathematical model obtained through the CCD-RSM was tested to evaluate its ability to make accurate predictions based on specific desired process outputs. To the best of our knowledge, this is the first study reporting the use of a CCD-RSM statistical approach to maximize the production of ethanol from non-sterilized sugar beet molasses while scaling up the experimental results up to a 100 L bioreactor scale.

Literature Research Gap

In order to implement an efficient fermentation process using industrial by-products, several process parameters such as sugar and yeast concentrations must be optimized to ensure high ethanol productivity while keeping in mind the economic aspects of the process (Darvishi et al., 2019). Nutrient supplementation is also an important parameter to take into consideration since an adequate amount of nutrient can significantly improve yeast viability and resistance to the medium, stimulating ethanol production performances. Since alcoholic fermentation is a complex biological process involving various operating factors, the use of the classical “one factor at a time” approach could be time-consuming due to the large number of experiments to perform. Hence, tools such as the statistical design of experiment (DoE) allow investigating the effect of several operational factors as well as their interactions on the overall process while considerably reducing the number of experimental tests (Keskin et al., 2016). For instance, a central composite design coupled with response surface methodology (CCDRSM) represents a powerful and effective statistical tool that could be

used for the optimization of biotechnological processes such as fermentation. Once developed, the CCD-RSM can be used to predict a process output, while imposing specific constraints based on economical and technical aspects. In this context, the present work aims to optimize the production of ethanol from non-treated sugar beet molasses produced by a local sugar refinery. For this purpose, a CCD-RSM was designed and developed to investigate the effect of three fermentation process parameters (initial sugar, yeast and nutrient concentrations) on ethanol productivity while considering several operating parameters such as ethanol yield and sugar utilization rate. Then, the second-order mathematical model obtained through the CCD-RSM was tested to evaluate its ability to make accurate predictions based on specific desired process outputs. To the best of our knowledge, this is the first study reporting the use of a CCD-RSM statistical approach to maximize the production of ethanol from non-sterilized sugar beet molasses while scaling up the experimental results up to a 100 L bio reactor scale

III. METHODOLOGY

The method adopted for this research work analysis is mixture data process analysis, using the design expert software to process the analyses.

3.1 Brewing Processes

The four basic ingredients in the brewing process are malt, water, yeast and hops. The central process is to extract the sugars from the malt grain so that the yeast can catalyze the decomposition reaction of sugars into alcohol (ethanol) and CO₂, which create the essentials of a beer. Even though every brewer has their own variations and preferred conditions within their process, they all follow a reliable framework for the brewing process. There are eight treatment processes in brewing shop which include the following: milling, mashing, wort separation, Wort Boiling, Wort Cooling and Aeration, Racking Process, Filtration Process and Packaging Process.

3.2 Beet Molasses Preparation

The beet molasses was collected from a local sugar refinery located in Sunti, Niger State and stored at 4 °C until further use. The sugar beet crops were cultivated and harvested by the same company before being processed to produce sugar. The fresh molasses was initially characterized for pH, density as well as for sugars and other metabolites concentrations.

3.3 Fermentation

After cooling, liquid yeast was transferred (6%, 8% and 10%) in Laminar flow chamber and placed in dark place for fermentation for a period of 14 days. After fermentation, fermented liquor was centrifuged at 5000 rpm for 15-20 min in order to remove all yeast cells. Supernatant was collected and stored in refrigerator at 4°C for further analysis.

3.4 Analytical Procedure

3.4.1 pH

The pH values of all samples were measured by digital pH meter of TOSCHON.

3.4.2 Titratable Acidity

Titrate acidity of fermented beverages was determined by the method of (Rangana, 2010), by using N/10 NaOH and expressed in term of malic acid.

3.4.3 Colour

Color was estimated calorimetrically according to (Daniels, 1995). Degassed sample was taken in 10 mm cuvette and absorbance was taken at 430 nm. Color was calculated by the formula given below.

Calculations:

$$\text{Color} = A \times f \times 25 \quad (3.1)$$

Where:

A is absorbance at 430 nm in a 10 mm cuvette and f is dilution factor.

3.4.4. Bitterness

Bitterness was estimated by the international method using ISO octane extraction and bitterness was given in Bitterness Units (BU). <http://dx.doi.org/10.1094/ASBCMOA-Beer-23>. Briefly, in 10.0 ml Transfer 10.0 ml chilled sample a minute amount of octyl alcohol, 1 ml 3N HCl (reagent b) and 20 ml 2,2,4-trimethylpentane was added and centrifuge for 15 min. As soon as possible, transfer sufficient clear, upper (iso octane) layer to cuvette of spectrophotometer and absorbance was taken at 275 nm with 2, 2, 4-trimethylpentane-octyl alcohol as blank.

Calculations:

Calculate bitterness units of beer by the formula,

$$BU = \text{absorbance } 275 \times 50. \quad (3.2)$$

3.4.5 Ethanol content

Ethanol content in fermented liquor was estimated by the spectrophotometric method of (Caputiet al., 1968). In brief, 1 ml of alcoholic sample was added directly to 30 ml with distilled water and then distilled at 70±2°C. 20 ml of distillate was collected in a 50 ml volumetric flask containing 25 ml of potassium dichromate solution. The contents in the volumetric flask were heated at 60°C in a water bath for 20 minutes and final volume was made to 50 ml with distilled water. After mixing and cooling the contents of the flask, the absorbance was recorded at 600 nm. The amount of ethanol in each sample was determined by using the standard curve of ethanol [0 – 20 % ethanol (v/v)].

Mathematically,

$$\text{Ethanol yield (Lkg}^{-1}\text{)} = \frac{\text{total liquid volume (L)} \times \text{ethanol concentration (vol\%)}}{\text{raw material weight (kg)}} \quad (3.3)$$

3.5 Signal-to-noise ratio for static designs

A signal-to-noise ratio is a measure of robustness, which can be used to identify the control factor settings that minimize the effect of noise on the response. Design Expert Software calculates a separate signal-to-noise (S/N) ratio for each

combination of control factor levels in the design. One can choose from different S/N ratios, depending on the goal of the experiment. In most cases it is desired to maximize the S/N ratio.

3.6 Orthogonal Array

Table 1: Experimental factors and their levels of the Production Quantities

Factor	Description	Level (L1)	Level (L2)	Level (L3)
A	Sugar Concentration(g/L)	125	225	325
B	Yeast Concentration(g/L)	0.2	0.6	1
C	Nutrient Concentration(g/L)	0	2	4

Table 2: DESIGN (ACTUAL)

		Compon ent 1	Compon ent 2	Compon ent 3	Respon se 1
I D	Ru n	A:S. Conc.	B:Y. Conc.	C:C	Alco. Yield (g/l/d)
2	1	324.571	1	2.42869	
1	2	325	1	2	
1	3	323.569	0.430978	4	
1	4	323	1	4	
6	5	324.087	0.838981	3.07382	
3	6	325	0.547105	2.45289	
6	7	324.087	0.838981	3.07382	
1	8	323.569	0.430978	4	
9	9	324.09	0.2	3.71011	
7	10	324.566	0.2	3.234	
3	11	325	0.547105	2.45289	
4	12	324.542	0.632311	2.8252	
8	13	323.524	0.950714	3.52492	

6	14	324.087	0.83898 1	3.07382	
5	15	324.944	0.2	2.856	
4	16	324.542	0.63231 1	2.8252	

IV. RESULTS AND DISCUSSIONS

4.1 Results

Table 3. BUILDING INFORMATION

File Version	13.0.5.0			
Study Type	Mixture		Subtype	Randomized
Design Type	I-optimal	Coordinate Exchange	Runs	16.00
Design Model	Quadratic		Blocks	No Blocks
Build Time (ms)	2909.00			

Table 4 : MIXTURE COMPONENTS

Component	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	S. Conc.		Mixture	323	325	+0 ↔ 323	+0.714286 ↔ 325	324.26	0.6144
B	Y. Conc.		Mixture	0.2	1	+0 ↔ 0.2	+0.285714 ↔ 1	0.6430	0.2932
C	C		Mixture	2	4	+0 ↔ 2	+0.714286 ↔ 4	3.10	0.6170
				Total =	328.00	L_Pseudo Coding			

Table 5 : RESPONSES

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	Alco. Yield	g/l/d	16.00	165	230	197.00	21.73	1.39

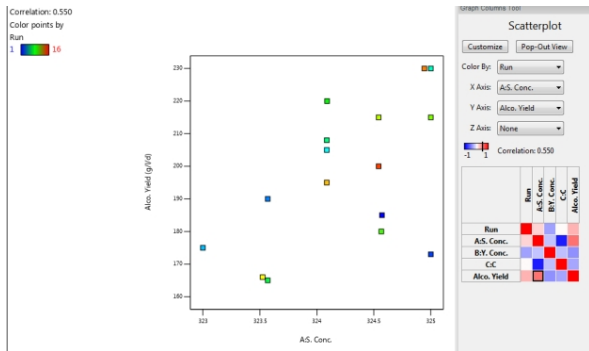


Figure 1: Scatter plot of Alcohol Yield against Sugar Concentration, Yeast Conc; and Nutrient Concentration.

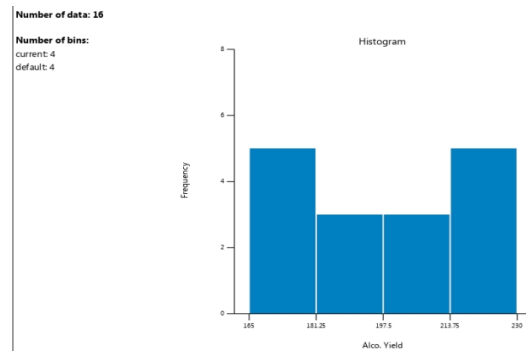


Figure 2: Graph of the Frequency Distribution of Alcohol Yield

Table 6: DESIGN CONSTRAINTS

Mixture Coding Actual

Low Limit		Constraint		High Limit
323.000	≤	A:S. Conc.	≤	325.000
0.200	≤	B:Y. Conc.	≤	1.000
2.000	≤	C:C	≤	4.000
		A+B+C	=	328.000

The table below was generated results of ethanol yield obtained through the experimentation data mixtures analysis as shown.

Table 7: Experimentation Data Mixtures Measurements Analysis

DESIGN (ACTUAL)

		Compon ent 1	Compon ent 2	Compon ent 3	Respon se 1
I D	Ru n	A:S. Conc.	B:Y. Conc.	C:C	Alco. Yield (g/l/d)
2	1	324.571	1	2.42869	185
1	2	325	1	2	173
1	3	323.569	0.430978	4	190
1	4	323	1	4	175
6	5	324.087	0.838981	3.07382	205
3	6	325	0.547105	2.45289	230
6	7	324.087	0.838981	3.07382	208
1	8	323.569	0.430978	4	165
9	9	324.09	0.2	3.71011	220
7	10	324.566	0.2	3.234	180
3	11	325	0.547105	2.45289	215
4	12	324.542	0.632311	2.8252	215
8	13	323.524	0.950714	3.52492	166
6	14	324.087	0.838981	3.07382	195
5	15	324.944	0.2	2.856	230

4	16	324.542	0.632311	2.8252	200
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Table 8: Fit Summary

Response 1: Alco. Yield
Mixture Component Coding is L_Pseudo

Source	Sequen tial p-value	Lac k of Fit p-value	Adjus ted R ²	Predic ted R ²	
Linear	0.0363	0.0877	0.3074	0.0109	Sugges ted
Quadr atic	0.2956	0.0872	0.3678	-0.3087	
Specia l Cubic	0.7442	0.0627	0.3063	-1.3332	
Cubic	0.0707	0.1540	0.6521	-101.2966	Sugges ted
Sp Quarti c vs Quadr atic	0.7401	0.0312	0.2365	-22.9930	
Quarti c vs Cubic	0.1540		0.7330		Aliase d
Quarti c vs Sp Quarti c	0.0312		0.7330		Aliase d

Table 9 : Sequential Model Sum of Square [Type I]

Response 1: Alco. Yield
Mixture Component Coding is L_Pseudo

Sourc e	Sum of Square s	d f	Mean Square	F- valu e	p- valu e	
Mean vs	6.209E+05	1	6.209E+05			

Total						
Linear vs Mean	2829.98	2	1414.99	4.33	0.0363	Suggested
Quadratic vs Linear	1266.04	3	422.01	1.41	0.2956	
Sp Cubic vs Quadratic	37.09	1	37.09	0.1133	0.7442	
Cubic vs Sp Cubic	1961.53	3	653.84	3.98	0.0707	Suggested
Quartic vs Cubic	355.19	1	355.19	2.82	0.1540	Aliased
Residual	630.17	5	126.03			
Sp Quartic vs Quadratic	461.34	3	153.78	0.4267	0.7401	
Quartic vs Sp Quartic	1892.47	2	946.24	7.51	0.0312	Aliased
Residual	630.17	5	126.03			
Total	6.280E+05	16	39251.50			

The highest order polynomial where the additional terms are significant and the model is not aliased will be the Sp Cubic - Cubic polynomial of the mean sum of square, 653.84; F-value, 3.98 and the p-value to be 0.0707

Table 10: ANOVA for Cubic model

Response 1: Alco. Yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6094.65	9	677.18	4.12	0.0493	significant
⁽¹⁾ Linear Mixture	2829.98	2	1414.99	8.62	0.0172	
AB	1283.37	1	1283.37	7.81	0.0314	
AC	1351.00	1	1351.00	8.23	0.0285	
BC	1333.25	1	1333.25	8.12	0.0292	
ABC	1265.95	1	1265.95	7.71	0.0321	
AB(A-B)	1228.61	1	1228.61	7.48	0.0340	
AC(A-C)	748.42	1	748.42	4.56	0.0767	
BC(B-C)	1521.98	1	1521.98	9.27	0.0227	
Residual	985.35	6	164.23			
Lack of Fit	355.19	1	355.19	2.82	0.1540	not significant
Pure Error	630.17	5	126.03			
Cor Total	7080.00	15				

The model F- value of 4.12 implies the model is significant. There is only 4.93 % chance of F-value of this large could occur due to noise.

P-value less than 0.0500 indicates model terms are significant. Values greater than 0.1000 indicate that the model is not significant. This mixture design process seems to be adequate as the model and other processes are significant.

The lack of fit F-value of 2.82 implies the lack of Fit is not significant relative to the pure error. Here, there is a 15.40% chance that a lack of fit F-value this large could occur due to noise. It is because we want the model to fit, therefore, the non-significant lack of fit is good.

Table 11: FIT STATISTICS

Std. Dev.	12.82	R ²	0.8608
Mean	197.00	Adjusted R ²	0.6521
As C.V. %	6.51	Predicted R ²	-101.2966
		Adeq Precision	6.3074
This negative	Predicted,	R ² , -101.2966 implies that the overall mean may be better predictor of the response than the current model	

Adequate Precision is a measure of the signal to noise ratio. A ratio greater than 4 is desirable. But the ratio 6.307 indicate adequate signal. The model can be used to navigate the designed space,

Table 12: MODEL COMPARISON STATISTICS

PRESS	7.243E+05
-2 Log Likelihood	111.33
BIC	136.29
AICc	159.33

Table 13 : COEFFICIENT IN TERMS OF CODED FACTORS

Component	Coefficient Estimate	d f	Standard Error	95% CI Low	95% CI High	VIF
A-S. Conc.	341.77	1	86.95	129.00	554.53	182.72
B-Y. Conc.	-28829.63	1	10241.78	-53890.37	-3768.90	3.609E+05
C-C	755.80	1	193.42	282.51	1229.08	724.11
AB	49832.63	1	17826.21	6213.46	93451.79	2.402E+05

AC	-1437.12	1	501.06	-2663.17	-211.08	562.86
BC	49881.11	1	17506.59	7044.02	92718.19	1.862E+05
ABC	-44783.64	1	16129.90	-84252.10	-5315.19	10723.05
AB(A-B)	-22924.67	1	8381.40	-43433.23	-2416.12	7685.88
AC(A-C)	728.24	1	341.13	-106.48	1562.97	25.23
BC(B-C)	26376.84	1	8664.40	5175.82	47577.86	5815.66

The coefficient estimate represent the expected change in response per unit change in factor value, when all the remaining factors remain constant. The intercept in the orthogonal design is the overall average response of all the runs. When the factors are orthogonal the VIF's is 1. VIF's greater than 1 indicate multi collinearity, so the more the VIF the severe correlation of factors. VIF's less than 10 are tolerable.

Final Equation in Terms of L_Pseudo Components
 Alco Yield = 341 A -28829.63 B +755.80 C +49832.63 AB -1437.12 AC +49881.11 BC -44783.64
 ABC - 22924.67 AB (A-B) +728.24 AC (A-C) + 26376.84 BC (B-C) (1)

Final Equation in Terms of Real Components

Alco. Yield = 44.136 S. Conc. - 7.334E+10 Y. Conc. + 2.291E+09 N. Conc. + 1.102 S. Conc. * Y. Conc. - 3.461E+09 S. Conc. * N. Conc. + 1.074 E+11 Y. Conc. * N. Conc. - 7.199E+10 S. Conc. * Y. Conc. * N. Conc. - 3.685E+10 S. Conc. * Y. Conc. * (S. Conc. - Y. Conc.) + 1.171E+09 S. Conc. * N. Conc. * (S. Conc. - N. Conc.) + 4.240E+10 Y. Conc. * N. Conc. * (Y. Conc. - N. Conc.) (2)

The equation in terms of the coded factors can be used to make prediction about the response for a given level of each factor. The higher the level of the mixture components are coded as +1 and the low levels are coded as 0. The coded equation is useful for identifying the relative impact of the factor by comparing the factor coefficients.

Final Equation in Terms of Actual Components

$$\text{Alco Yield} = 0.136 \text{ S. Conc.} - 2.236 \text{ E}+08 \text{ Y. Conc.} + 6.984\text{E}+06 \text{ N. Conc.} + 1.024\text{E}+06 \text{ S. Conc.} * \text{Y. Conc.} - 32171.379 \text{ S. Conc.} * \text{N. Conc.} + 9.984 \text{ E}+05 \text{ Y. Conc.} * \text{N. Conc.} - 2040.071 \text{ S. Conc.} * \text{Y. Conc.} * \text{N. Conc.} - 1044.309 \text{ S. Conc.} * \text{Y. Conc.} * (\text{S. Conc.} - \text{Y. Conc.}) + 33.174 \text{ S. Conc.} * \text{N. Conc.} * (\text{S. Conc.} - \text{N. Conc.}) + 1201.569 \text{ Y. Conc.} * \text{N. Conc.} * (\text{Y. Conc.} - \text{N. Conc.}) \quad (3)$$

The equation in terms of the coded factor can be used to make prediction about the response for a given level of each factor. The level should be specified in the original units for each factor and the average value of the Alcohol yield gives the optimal yield.

2.1.2: DIAGNOSTIC

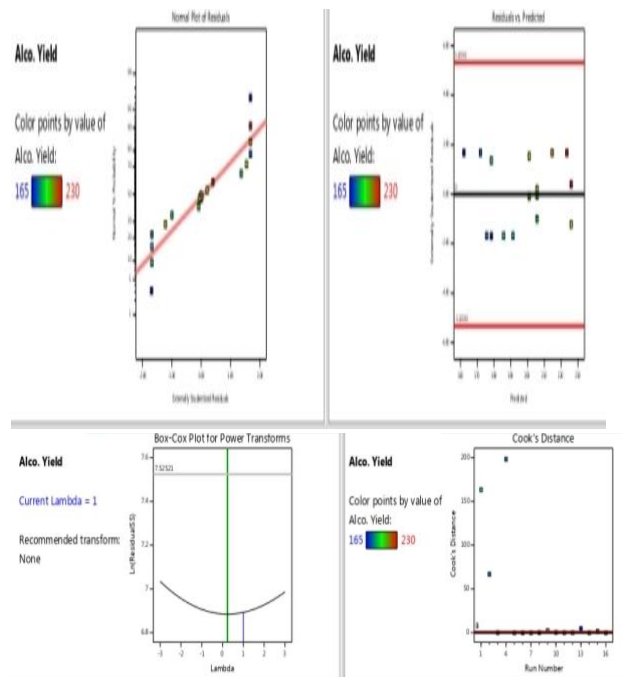


Figure 3: Graph of Diagnostics

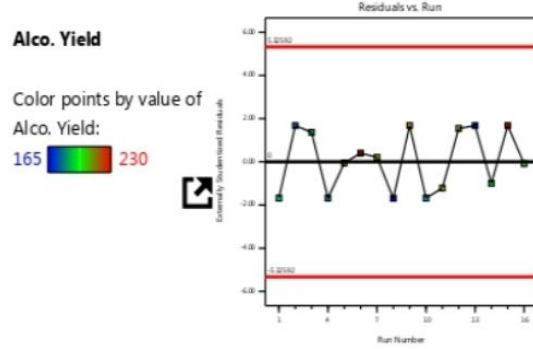


Figure 4: Graph of Residual Vs Run

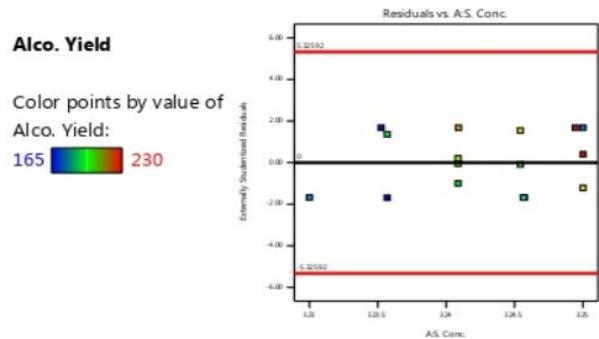


Figure 5: Graph of Externally Studentized Residuals Vs Sugar Concentration

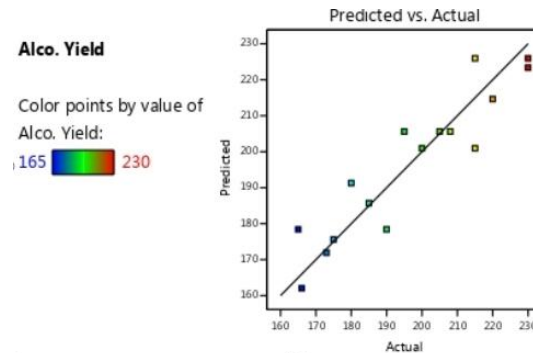


Figure 6: Graph of Predicted Vs Actual Alcohol Yield

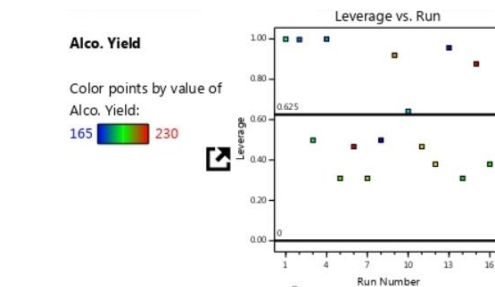


Figure 7: Graph of Leverage Vs Run Number

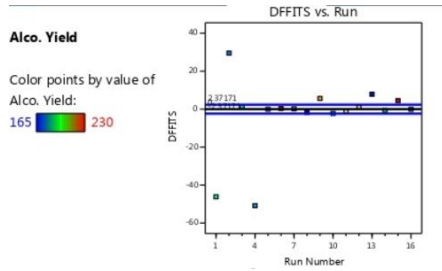


Figure 8: Graph of DFFITS Vs Run Number

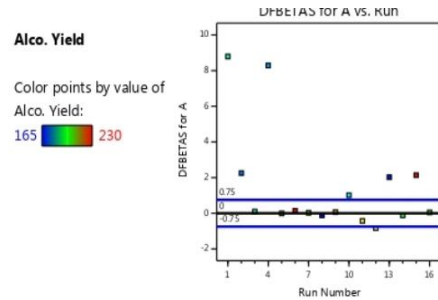


Figure 9: Graph of DFBETAS for A Vs Run Number

Table 14 : REPORT

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	185.00	185.68	-0.6849	0.999	-1.471	-1.679	163.537 ⁽¹⁾	-46.162 ⁽¹⁾	2
2	173.00	171.93	1.07	0.997	1.471	1.679	66.946 ⁽¹⁾	29.535 ⁽¹⁾	1
3	190.00	178.41	11.59	0.498	1.276	1.365	0.161	1.358	15
4	175.00	175.62	-0.6219	0.999	-1.471	-1.679	198.421 ⁽¹⁾	-50.848 ⁽¹⁾	16
5	205.00	205.60	-0.6018	0.309	-0.056	-0.052	0.000	-0.035	8
6	230.00	225.95	4.05	0.466	0.432	0.401	0.016	0.375	4
7	208.00	205.60	2.40	0.309	0.225	0.206	0.002	0.138	9
8	165.00	178.41	-13.41	0.498	-1.477	-1.689	0.216	-1.681	14
9	220.00	214.64	5.36	0.919	1.471	1.679	2.453 ⁽¹⁾	5.653 ⁽¹⁾	13
10	180.00	191.29	-11.29	0.641	-1.471	-1.679	0.386	-2.244	11
11	215.00	225.95	-10.95	0.466	-1.170	-1.216	0.120	-1.137	3
12	215.00	200.94	14.06	0.379	1.392	1.544	0.118	1.206	6
13	166.00	162.05	3.95	0.956	1.471	1.679	4.713 ⁽¹⁾	7.837 ⁽¹⁾	12
14	195.00	205.60	-10.60	0.309	-0.995	-0.994	0.044	-0.665	10
15	230.00	223.37	6.63	0.876	1.471	1.679	1.531 ⁽¹⁾	4.467 ⁽¹⁾	7
16	200.00	200.94	-0.9408	0.379	-0.093	-0.085	0.001	-0.066	5

Box-Cox Power Transformation

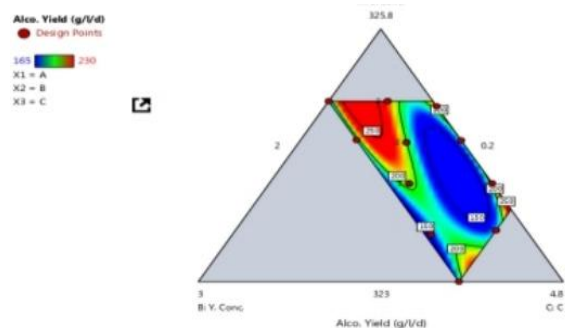


Figure 10: Model Graph of Component Actual Coding

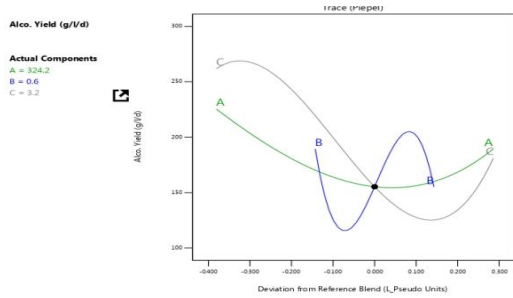


Figure 11: Graph of Alcohol Yield against Deviation from Reference Blend (L_Pseudo Units)

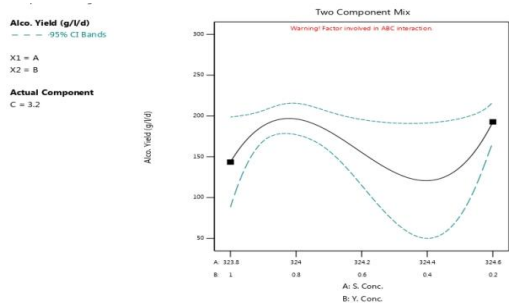


Figure 12: Graph of Alcohol Yield against Two Component Mix (Sugar Concentration and Yeast Concentration)

2.1.3. Optimization

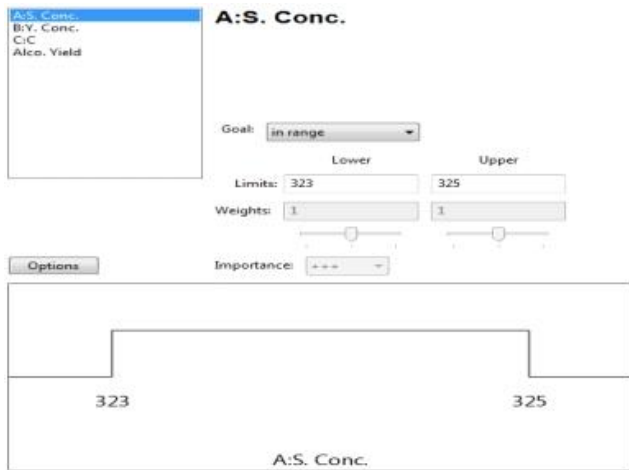


Figure 13: Graph of the Optimization Criteria of Alco. Yield Vs Sugar Conc.

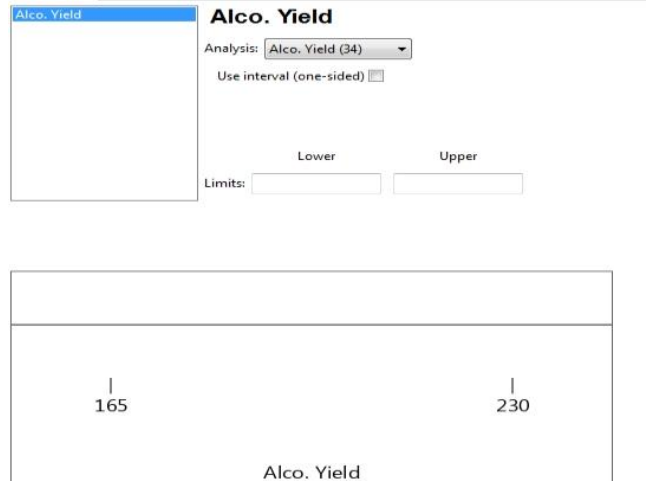


Figure 14: Graph of the Optimization Criteria of Alco. Yield .

2.1.4. Post Analysis

Table 15: Factors

Component	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	S. Conc.	324.20	323.00	325.00	0.00	Actual
B	Y. Conc.	0.60	0.20	1.00	0.00	Actual
C	C	3.20	2.00	4.00	0.00	Actual
	Total=	328.00				

Table 16 : Confirmation of Analysis Result

Two- sided confidence = 95% Population = 99%

Analysis	Predicted mean	Predicted median	Observ.	Standard Dev.	SE Mean	95% CI low	95% CI high	95% TI Low	95% TI High
Alco Yield	155.328	155.328		12.815	16.479	115.004	195.651	55.494	255.162

Table 17: Confirmation Point of mixture Analysis

S. Conc.	Y. Conc.	C
324.2	0.6	3.2

Response data

Runs:

Table 18: Coefficients Table

p-value shading: **p < 0.05** 0.05 ≤ p < 0.1 p ≥ 0.1

	A	B	C	AB	AC	BC	ABC	AB(A-B)	AC(A-C)	BC(B-C)
Alco Yield	341.8	- 28829.6	755.8	49832.6	- 1437.12	49881.1	- 44783.6	- 22924.7	728.244	26376.8
p-values				0.0314	0.0285	0.0292	.0321	0.0340	0.0767	0.0227

From table 17 of the mixture components in the analysis were substituted into the final equation in Terms of actual components and evaluated the alcohol yield as given below:

Alcohol Yield = - 1436800475.25 g/l/d. This is the volume of the optimized alcohol yield per gram per litre drum.

2.2 Discussion of Results

From the table 7,: experimentation data mixtures analysis is the orthogonal data mixture for Alcohol yield experimental analyses were generated, from which analyses of the mixtures tool design analysis

with the Design of experiment software was used to test for significance of the factors in the analysis

found in both the tables and figures as presented in this work.

From Table 10 ANOVA for Selected mixture Model; Response 1: Alcohol Yield showed the model terms generated are significant.. The model is separated into individual terms and tested independently and observed to be all significant and residual unaccountable. The model F- value of 4.12 implies the model is significant. There is only 4.93 % chance of F-value of this large could occur due to noise.

P-value less than 0.0500 indicates model terms are significant. Values greater than 0.1000 indicate that the model is not significant. This mixture design process seems to be adequate as the model and other processes are significant.

The lack of fit F-value of 2.82 implies the lack of Fit is not significant relative to the pure error. Here, there is a 15.40% chance that a lack of fit F-value this large could occur due to noise. It is because we want the model to fit, therefore, the non-significant lack of fit is good.

From table 11, the Fit statistics terms presented are: Std. Dev. 12.82 is an estimate of the standard deviation associated with the experiment Mean of 197.00 is an overall average of the response data (Alcohol yield) Coefficient of Variation C.V. % of 6.51 is the standard deviation expressed as a percentage of the mean. Calculated by dividing the Std Dev. by the Mean and multiplying by 100.

R squared, R^2 0.86 is a measure of the amount of variation around the mean explained by the model. Adjusted R^2 0.652 is a measure of the amount of variation around the mean explained by the model, adjusted for the number of terms in the model. The adjusted R-squared decreases as the number of terms in the model increases if those additional terms don't add value to the model.

Predicted R^2 -101.296 is a measure of the amount of variation in new data explained by the model. Adequate Precision 6.307 is a measure of signal-to-noise ratio. It compares the range of the predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination.

This negative predicted R^2 , -101.297 implies that the overall model response is better than the current model.

Adequate Precision is a measure of the signal to noise ratio. A ratio greater than 4 is desirable. But the ratio 6.307 indicate adequate signal. The model can be used to navigate the designed space,

From the table 12, Model Comparison Statistics terms as:

PRESS: Predicted Residual Error Sum of Squares of $7.24E+05$ is a measure of how the model fits each point in the design. The PRESS is computed by first predicting where each point should be from a model

that contains all other points except the one in question. The squared residuals (difference between actual and predicted values) are then summed.

-2 Log Likelihood: - 111.33, this is derived by iteratively improving the coefficient estimates for the chosen model to maximize the likelihood that the fitted model is the correct model. For balanced, orthogonal designs, this is exactly the same result as least squares regression. The -2 log likelihood is used to compute the following penalized modeling statistics.

BIC 136.29 a large design penalized likelihood statistic used to choose the best model.

AICc: 159.33, a small to medium (most designs) penalized likelihood statistic used to choose the best model.

From the table 18: Coefficients in Terms of Actual Factors,

Coefficient Estimate: is the Regression coefficient representing the expected change in response Y per unit change in X when all remaining factors are held constant. In orthogonal two-level designs, it equals one-half the factorial effect.

Standard Error: the standard deviation associated with the coefficient estimate of each term as shown in the table.

95% CI High and Low: If this range spans 0 (one limit is positive and the other negative) then the coefficient of 0 could be true, indicating the term is not significant. Hence all are significant from the table 13.

VIF: Variance Inflation Factor – Measures how much the variance around the coefficient estimate is inflated by the lack of orthogonality in the design. If the factor is orthogonal to all other factors in the model, the VIF is one. Values greater than 10 indicate that the factors are too correlated together (they are not independent.)

Model developed in this research that predict the alcohol yield using the data point of table 17, confirmation location was shown in equation (1, 2,

and 3). According to the R-square value, the model predict the alcohol yield with high precision. Evaluation of the equation with the confirm point in the mixture terms gave the model mean value of 197.00.

CONCLUSIONS

It is now crystal clear that alcohol beer yield is feasible in production with the spent grain from beer waste when upgraded with beet molasses. The average volume of alcohol yield has become 1436800475.25 g/l/d which was considerably good, considering the range of 323 and 325 sugar concentration; 165 and 230alcohol yield as were experimentally determined. In the same way the analysis of variance from table 10, were observed that the associated terms were significant.

Model developed in this research that predicted the alcohol yield using the data point of table 17, confirmation location point was shown in equation (3). According to the R-square value, the model predicted the alcohol yield with high precision. Evaluation of the equation with the mixture terms gave the model mean value of 195.00 g/ l/d. of the fit statistics table.

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