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Effects Of Process Factors On The Synthesis Of Bioethanol From Cassava Tubers Using H_2S0_4 As Catalyst.

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--ABSTRACT--
The Separate hydrolysis and fermentation method of ethanol production from cassava tubers using sulfuric acid as catalyst for the hydrolysis reaction was studied. Dry milling process was specifically used for the flour activation prior to the hydrolysis process. Two level full factorial design was used to study the effects of three process factors on the ethanol yield. The factors studied were fermentation time (days), particle size (μm) and strength of acid (M). It showed that fermentation time and particle size had significant effects on the yield with effect of strength of acid being marginal. The diagnosed linear model obtained showed that it can explain the process well. The optimum condition was obtained at fermentation time of five days, particle size of 300μm and strength of acid of 1M with predicted response of 25.3945%V/V of ethanol concentration at the desirability of one.

Keywords: *ANOVA. Cassava, Ethanol, Factorial design, Optimization .*

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I. INTRODUCTION

Ethanol and ethanol-gasoline mixture has been considered for use as fuel since the early days of automobile. In the past, the abundant and less expensive petroleum supply prevented the extensive use of ethanol as fuel, but in the last few decades, the general public has become aware of and concerned about the increasing expensive petroleum supply [1]. Ethanol has been produced by anaerobic yeast fermentation of simple sugars since early recorded history. These fermentations used the natural yeast found on fruits and the sugars of these fruits to produce wines [1]. Current practices utilize bacterial and fungal analysis to efficiently hydrolyze grain or tuber starch to glucose for fermentation to ethanol [2].

The profitability of fuel ethanol production is crucially determined by cost of feedback used. The feedback cost typically represent more than 5% of the total production cost, and is the drilling factor for researching the potentials of low cost lignocelluloses' biomass for ethanol fermentation [3]. After feedback costs, energy cost for ethanol fermentation is about 30% of the total production cost [4].Ethanol can be produced by biologically catalyzed reactions. For starch crops, such as corn, cassava tubers, etc, starch is first broken down to simple glucose sugar by acids or enzymes known as amlyses. Acids or cellulose enzymes similarly catalyze the breakdown of cellulose into glucose, which can be then fermented to ethanol [1].Enzyme hydrolysis for the production of ethanol is an expensive process for the production of alcohol from starch materials [5]. Chemical hydrolysis gives advantages for short residence time than enzyme hydrolysis [5].Basically, two different processes can be used to produce ethanol from starchy crops; dry milling and wet milling processes. In dry milling, the feed material is ground mechanically and cooked in water to gelatinize the starch. The enzymes or acids are then added to breakdown the starch to form glucose, which yeast ferments to ethanol. In wet milling, the insoluble protein, oil, fiber and some solids are removed initially, remaining only slurry of starch fed to the ethanol production step.The separate hydrolysis and fermentation process uses distinct process steps for starch hydrolysis and glucose fermentation. The primary advantage of this process is that, hydrolysis and sugar fermentation can be treated separately, thus minimizing the interaction between these steps [1].Cassava (manihot esculenta), sometimes called manioc, is third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tones [6]. On infertile land where the cultivation of other crops is difficult, unless considerable inputs are applied, cassava still has reasonable yield. The edible parts are the tuberous roots and leaves. The tuber (root) is somewhat dark brown in color and grows up to 2 feet longs [6].

Cassava tuberous roots can be processed into many different products due to their high (28-35%) starch content [7]. All these will promote a great increase in the growing of cassava. Cassava has the following advantages with respect to its utilization in the starch industry;

[1] Low production cost
[2] Can be planted in poor soil
[3] Excellent starch quality for its white, sticky and lucent characteristic. According to statistical
data, total national starch production reached 5million tones/year, of which 11% is cassava starch, 80% maize starch and 9% starch from other crops [7]. Large quantities of cassavas are been produced but despite a consideration effort, there is at present no commercially proven glucose syrup or alcohol from cassava industry [8]. Cassava flour promises to be a good substrate for alcohol production due to its high content of fermentable sugar and stable shelf-life [9, 10]. Besides that, it has advantages such as complete and easier hydrolysis compared to other flours [11].The aim of this work was to produce bio-ethanol form cassava tubers using separate hydrolysis and fermentation process thus, minimizing the interaction between the steps, to study the effects of three process variables on the ethanol yield, to obtain the model equation and to optimize it.

II. MATERIALS AND METHODS

2.1 Materials

Cassava tubers were purchased from Abakpa Market at Enugu, in Enugu State of Nigeria. The tubers were peeled and thoroughly washed to reduce the cyanide content, sliced into small pieces, dried under the sun, ground to flour and pass through sieves of 180μm and 300μm sizes. It was stored in a dry plastic container throughout the experiment. Baker's yeast (Saccharomyces cereviseae) was purchased from De-cliff integrated at Ogbete market in Enugu, Enugu State Nigeria. Double distilled water was obtained from Pymotech research center and laboratory at Abakpa in Enugu State Nigeria. The sulfuric acid used was of Analytical grade.

Pretreatment

The dry milling process was used before the acid activation, this step enhanced visibility of the pores of the flour for easier hydrolysis by the acid. In this method, the sieved cassava flour was boiled in water with constant stirring to a temperature of $70^{O}C$ until the flour gelatinized.

Acid Hydrolysis

To the gelatinized sample, the prepared solution of the sulfuric acid was added with constant stirring until a homogenous mixture was formed. The solution was heated with constant stirring on a magnetic stirring hotplate until temperature of $65^{O}C$ was reached. At this point, the texture and color of the solution changed. This was allowed to cool, filtered through No.1 whatman filter paper and the pH was adjusted to 4.5 with 0.1M Na0H.

Fermentation

The cassava flour hydrolysate was fermented in an aspirator bottle (previously sterilized to exclude other microorganisms) with the baker yeast. The bottle was topped with straw to allow carbon dioxide to escape. Fermentation was done for 5 days and 8 days at room temperature. At the end of the fermentation period, the alcohol was separated from the extract using simple distillation.

Distillation process

The distillation apparatus consisted of conical flask, condenser, splash head (to avoid the entrance of water vapors into the receiver) and the receiver. The fermented cassava flour hydrolysate was added into the conical flask and the set up was heated with a heating mantle at temperature between $75\text{-}80^{O}C$. The distillate collected was allowed to cool and the density was obtained.

Determination of Percentage Ethanol Concentration

A slight modification of method according to Oyeleke and Jibrin 2009 [12] was used. Series of percentage (V/V) ethanol water solution were prepared and were weighed. The density of each of the prepared ethanol solution was calculated and a standard curve of density against percentage ethanol was plotted. The distillate was weighed and its density calculated. The percentage ethanol concentration of ethanol produced was obtained by comparing its density with the standard ethanol density curve.

Experimental design

Two level full factorial design was used to obtain both the single and interaction effects of the process. An experiment is called factorial experiment if the treatments consist of all possible combinations of several levels of the factors. It reveals the effect of interaction of process variables and improves process optimization [13]. The process variables considered in this study were particle size (μm), strength of acid (M) and fermentation time (days). The factors and level for the full factorial design used for the experiment is shown on table I below.

Table 1 Factors and Levels for 2-Levels Full Factorial Design

FACTORS	UNITS	LOW LEVELS	HIGH LEVELS
Strength of acid	M	1	3
Particle size	μm	180	300
Fermentation time	Days	5	8

The experimental runs were randomized to satisfy the statistical requirement of independence of observation [13]. Randomization acts as insurance against the effect of lurking time-related variables [14].The design layout with both experimental and predicted values by the model is shown on table 2 below.
The condition of the experiment was based strictly on the design layout.

Table 2: Design Layout with both actual and predicted values

Std Order	Run Order	Particle size (μm)	Acid concentration (M)	Fermentation time (Days)	Experimental value (%V/V)	Predicted value (%V/V)
1	1	180.00	1.00	5.00	16.00	159.94
6	2	180.00	3.00	8.00	6.00	6.06
4	3	300.00	3.00	5.00	16.76	15.91
3	4	300.00	1.00	5.00	25.33	25.39
8	5	300.000	3.00	8.00	15.50	15.52
5	6	180.00	1.00	8.00	9.00	8.15
2	7	180.00	3.00	5.00	12.35	13.20
7	8	300.00	1.00	8.00	10.00	10.85

III. RESULTS AND DISCUSSIONS

The percentages of ethanol yield were compared by varying three factors at different combination of their levels using two levels full factorial design. The design expert 8.0.7.1 was used for the analysis of the data. The factors studied were: A. strength of Acid (M), B. particle size (μm), and C. fermentation time (days). The factors that were included in the model were selected based on the half normal plot in fig. 1 below.

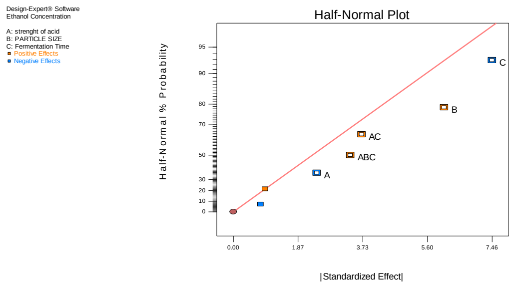

Fig 1. Half normal plot

From the figure, it showed that fermentation time (C), particle size (B), interaction effect of Acid Strength and fermentation time (AC), interaction effects of the three factors (ABC), and lastly, strength of acid (A) were significant. They were displayed according to the magnitude of their effects.

3.1 ANOVA

The F-test analysis of variance (ANOVA) was used to check the statistical significance of model equation. Values of probability > F indicate that model terms are significant. From the ANOVA table on table 3 below, the model F-value of 33.88 implied the model was significant. There is only a 2.89% chance that a "model F-value" this large could occur due to noise. Values of probability greater than 0.100 indicated the model terms were not significant. In this case, B, C, and AC were significant model terms. The goodness of the model can be checked by different criteria. Fischer's F-test indicates the overall significance of a model and its associated probability P, correlation coefficient R, and coefficient of determination R^2 measure the goodness of fit of regression model [3].

Table 3 ANOVA for Selected Factorial Model

Source	Sum of Squares	df	Mean squares	F value	P-value Prob > F
Model	247.18	5	49.44	33.88	0.0289
Strength of Acid (A)	11.59	1	11.59	7.94	0.1062
Particle Size (B)	73.96	1	73.96	50.68	0.0192
Fermentation time (C)	111.42	1	111.42	76.35	0.128
AC	27.40	1	27.40	18.78	0.0494
ABC	22.82	1	22.82	15.64	0.0584
Residual	2.92	2	1.46		
Cor total	250.10	7			

The value of R^2 was 0.9883, indicating that about 1.19% variation was not explained by the model. The value of adjusted R^2 was high (0.9592) to advocate high significance of the model. The value of coefficient of variation (CV = 8.70) was low due to small residue between actual and predicted values. Adequate precision measures signal to noise ratio, a value of 18.477 obtained indicated a better precision, the reliability of the experiments carried out and an adequate signal to use the model for prediction purposes [15].

The model equation obtained in terms of the coded factor is

$$Y = +13.88 - 1.20A + 3.04B + 3.73C + 1.85AC + 1.69\,ABC.$$

Where, Y is the ethanol concentration in % (V/V).

Validation of model

To verify the assumptions made by ANOVA, the model was validated using diagnostic plots.

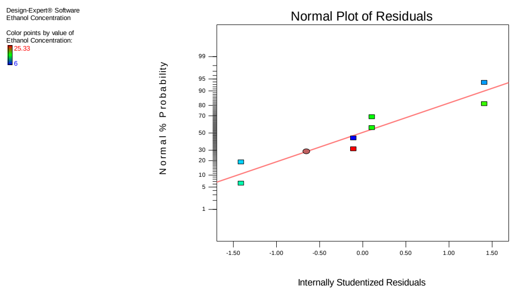

Fig 2a. Normal plot of residuals

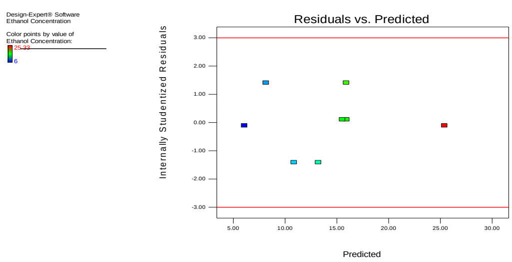

Fig 2b. Plot of residual vs. predicted values

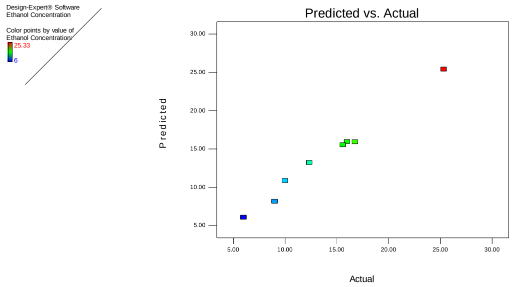

Fig 2c. Plot of predicted vs. actual values

The diagnostic plots in figure 2 above revealed no problem, showing that there was a good correlation between the experimental values and values predicted by the model.

One factor effect

The model graphs were used to study the single effects of the factors.

Effect of Strength of Acid

The effect of Acid strength on the ethanol yield was studied for acid strength of IM and 3M. On acid hydrolysis, the sulfuric acid used acted as a chemical catalyst for the breakdown of cellulose into glucose which was fermented to ethanol. It is a known facts that increase in the concentration of the Acid speeds the rate of hydrolysis of flour. But as was seen on this study, the analysis of variance showed that the increase in concentration of sulfuric acid from IM to 3M had F Value of 7.94 with probability > F of 0.1062. It showed that it was marginally significant. This was depicted in the graph in figure 3 below.

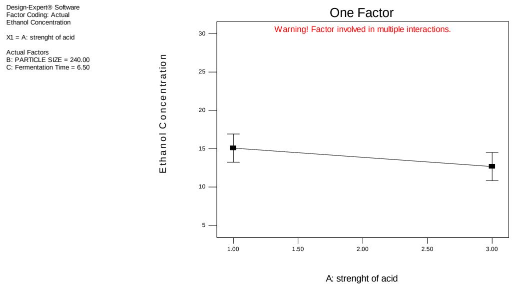

Fig 3. Effect of strength of acid

In order words, increasing the concentration of acid to IM was very sufficient to allow the hydrolysis of the flour. To save cost, it is better to use a lower concentration of 1M to hydrolyze since acid concentration had a marginal effect on the ethanol yield.

Effect of Particle Size

The particle size effect was studied using particle size of 180μm and 300μm. From the half normal plot, it showed that particle size was second in the order of the effect which showed how important it is in ethanol production. From the plot in figure 4, it showed that it had a positive effect on the ethanol yield.

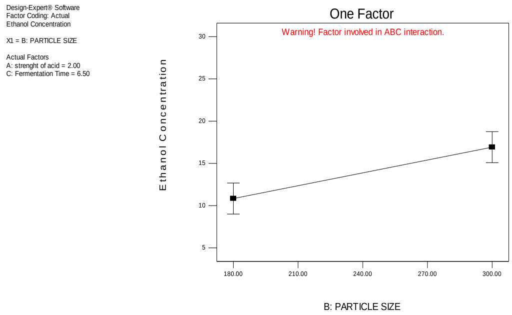

Fig 4. Effect of particle size

The finer the particle size, the larger is the area that can be attacked by the catalyst. Moreover, the constituents of the flour hydrate faster for fine particle size, and this makes them more readily accessible to the catalysts.

Effect of time of fermentation

The effect of time of fermentation in days was studied at 5 days and 8 days. From the half normal plot and analysis of variance table, it was observed that fermentation time had highest effect which was negative. From the graph, in figure 5 below, it showed a negative graph meaning that the highest ethanol yield was obtained at the 5th day.

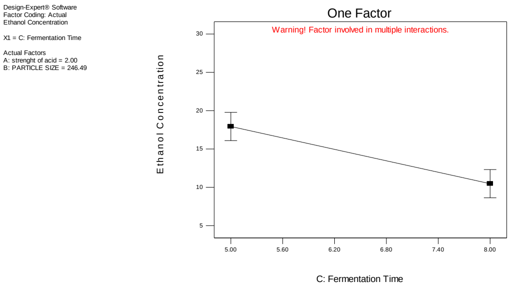

Fig 5. Effect of fermentation time

This was in line with a wok done by [12, 16], where they reported that the maximum ethanol yield from guinea corn husk, millet husk and fresh fruit was obtained at the 5th day. Fermentation above the 5th day decreased the ethanol yield.

Interaction Effects

One advantage of the factorial design analysis is that, it will give both the single and interaction effects of the factors without aliases. The interaction effects were studied using line, contour and 3D surface plots. From the ANOVA table, the interaction effect of strength of Acid and fermentation time (AC) had effect and also the interaction effects of the three factors studied (ABC).

Interaction effects of strength of acid and fermentation time.

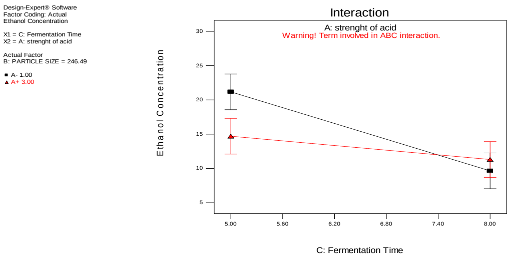

Fig 6. Interaction effect of strength of acid and fermentation time

From the above figure, the red line is the high level of the strength of acid, while the black line shows the low level of the strength of acid. From the plot, it showed that at the high level of the strength of acid, fermentation time had little effect on the ethanol yield. While at the low level of the strength of acid, increase in the fermentation time from five to eight days led to the decrease in ethanol yield.

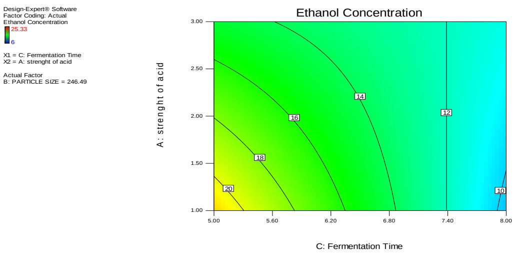

Fig 7. Contour plot of interaction effect of strength of acid and fermentation time

From the contour plot above, it showed that higher ethanol concentration was obtained at lower fermentation time and reduced strength of acid.

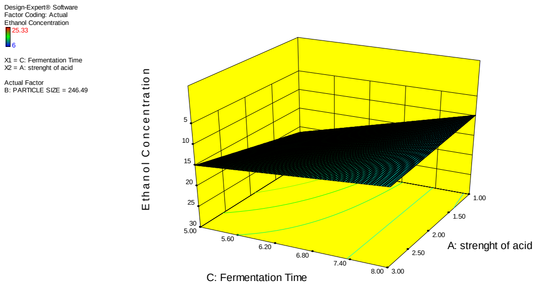

Fig 8. 3D surface plot

The 3D surface plot also confirmed the findings of contour plot.

Interaction effect of strength of acid and particle size

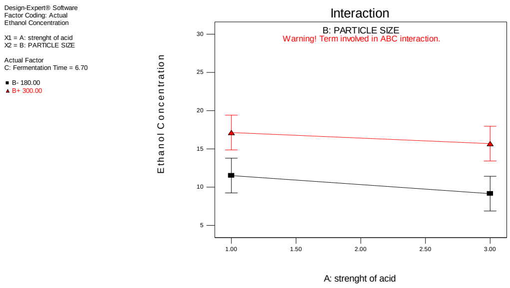

Fig 9. Interaction effects of strength of acid and particle size.

The interaction effects of strength of acid and particle size was also analyzed. From the plot in figure 9 above, it showed that at both high and low levels of the particle size, the increase in strength of acid had little or no effect on the ethanol yield.

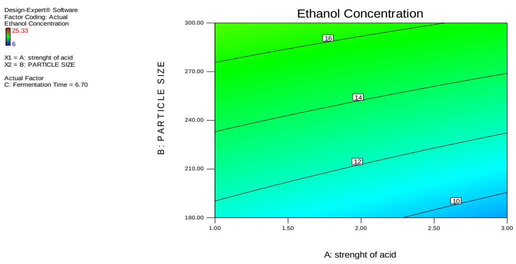

Fig 10. Contour plot

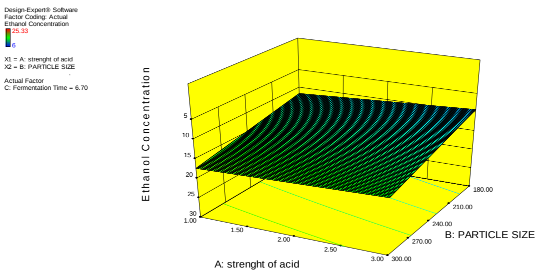

Fig 11. 3D surface plot

The contour and 3D surface plots showed that increase in ethanol yield was obtained with high particle size and low strength of acid.

Cube Plot

The cube plot was used in estimating the three factor interaction effect. Since the interaction effects of the three factors were significant, the cube analysis became paramount.

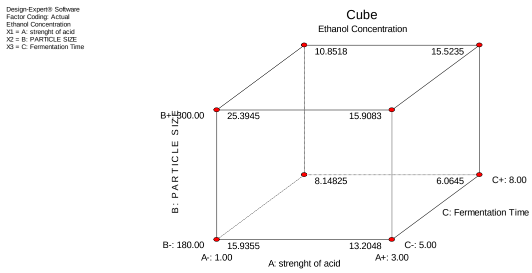

Fig 12. Cube plot of the interaction effects of the three factors

From figure 12 above, the highest ethanol concentration (25.3945%v/v) was obtained at the upper front left corner at the factor setting of B+, C-, and A- and was lowest at (6.0645%v/v) at the lower back right corner at the factor setting of C+, B+, and A+. Where minus signs meant the factor at its lower level and positive signs meant the factor at its high level.

Process optimization

Once a good model was obtained, it can be optimized. The numerical optimization done with maximization of ethanol yield as the goal, gave 23 solutions. According to the software, the best condition out of the twenty-three solution will be based on the desirability. The optimum conditions selected were at desirability of one. A desirability of one means goal was easy to reach and one can probably do better [17]. With this, the optimum conditions were: strength of Acid of 1M, particle size of 300μm and fermentation time of 5 days giving ethanol concentration of 25.3945% V/V.

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IV. CONCLUSION

The 2 level full factorial design (2^3) was applied on the ethanol production from cassava tubers using acid catalyst. The factorial design was used to find the factors that were significant, their interaction effect, model equation that will explain the process well and finally optimized the model using numerical optimization tool. The study revealed that fermentation time had highest effect on the ethanol concentration, followed by the particle size and lastly, the concentration of Acid which had marginal effect. Equally, it revealed that the interaction effect of fermentation time and concentration of Acid, and the three factors interaction effect were significant too. The validated model equation obtained showed that it can explain the process well under the same condition of the experiment. The optimum conditions obtained were: fermentation time of 5 days, particle size of 300μm and strength of Acid of 1M with predicted response of 25.3945% V/V of ethanol concentration at the desirability of 1.The result revealed that ethanol could be produced from cassava tubers using sulfuric acid as the catalysts for the hydrolysis step.

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