OPTIMIZATION OF PROCESS PARAMETERS IN ETHANOL PRODUCTION FROM CASSAVA WASTE SLURRY

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ABSTRACT

The production of ethanol from renewable material is a sustainable avenue of ethanol production. This study focused on optimizing parameters affecting the production of ethanol generated from cassava waste slurry. The waste generated from cassava were characterized using proximate analysis. The proximate analysis results showed that the cassava waste slurry contain more moisture, volatile matter and fixed carbon. Central composite experimental design (CCD) was used to design and model the process with 50 experimental runs. CCD, with quadratic models explored the combined effect of five independent variables namely, temperature, PH, sugar concentration, time, and feed rate of the fermenting medium. The process parameters were optimized to obtain the optimal yield, purity and specific gravity. The experimental result showed that the maximum ethanol yield of 26% was obtained at a temperature of 40^0C, pH of 4, sugar concentration of 0.125, production time of 0.5hrs and feed value of 250ml. The statistical analysis of the yield, purity and specific gravity showed correlation coefficient (R^2) of 0.88, 0.91 and 0.82 respectively. The effect of the process parameters showed that increase in the reaction temperature, feed, time, and pH increases the yield of ethanol while increase in the sugar concentration decreases the ethanol yield. The optimization result showed that the optimal yield of 10.54%, purity of 5.76% and specific gravity of 0.26 were obtained at reaction temperature of 30.06 ℃, pH of 5.40, sugar concentration of 0.30ml/dm^3 and reaction time of 1.03 hours.

Keywords: *Cassava, Fermentation, Hydrolysis, Optimization, Yield*

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1.0 Introduction

Recently, researchers have considered it important to reinforce the development in the energy sector. One energy source that is sparingly mentioned in national projects and has demonstrated its feasibility in other regions of the world is the production of ethanol (Leen *et al*., 2007).

Ethanol, chemically known as ethyl alcohol, is the second member of the aliphatic alcohol series; it is a clear, volatile, flammable, colorless liquid with an agreeable odor. It can be detected in traces of free State or in form of its esters as it is .produced in plant and animals by decomposition of complex organic compounds (Ugwu and Omoruyi, 2016). Cassava (*Manihot esculenta*) sometimes also called manioc or tapioca root is a di-cotyledons plant, 1-3 meters height when fully grown and It belongs to the family of Euphorbiaceous (Osagie,1998).

Cassava plant probably originated in Brazil, which is regarded as the leading world producer followed closely by Indonesia. Since 1990, Nigeria has surpassed Brazil as the world's leading producer of cassava with an estimated annual production of 26 million tons from an estimated area of 1.7million hectares of land. Other major producers of cassava are Zaire, Thailand, China, Indonesia, Malaysia, Malawi, Togo, and Tanzania (Akinola, 2007). Cassava is highly efficient in producing starch and it is tolerant to extreme stress conditions. Furthermore, it fits nicely within traditional farming systems and its fresh roots contain about 30% starch. Cassava starch is one of the best fermentable substances for ethanol production.

Fermentation is the oldest way for humans to produce ethanol, and this is also the traditional way of making alcoholic beverages (Leen *et al*., 2007). Ethanol can be produced from biomass by the hydrolysis process and followed by sugar fermentation processes. Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi cellulose and lignin. To produce sugars from the cassava, the cassava is pre-treated with acids or enzymes to reduce the size of the feedstock and to open the plant structure (Saoharit *et al*., 2009). The cellulose and the hemi cellulose portions are broken down (hydrolysed) by enzymes or dilute acids into sucrose sugar that is then fermented into ethanol. There are three principle methods of extracting sugars from cassava. These are concentrated acid hydrolysis, dilute acid hydrolysis and enzymatic hydrolysis (Akihiko *et al*., 2008). Previous studies evaluated the

environmental impacts of bio-based fuels in various categories, including non-renewable energy consumption, greenhouse gas emissions, acidification, eutrophication, human and ecological health, and photochemical oxidation. Most studies have concluded that the use of ethanol as liquid fuel could reduce greenhouse gas emissions among economics, environmental impacts, and energy for the most effective use of regional energy resources (Hu *et al*., 2004).
Production of ethanol for bio-fuel will reduce the demand for petroleum as a source of energy for powering engine and also reduce environmental pollution of petrol through the emission of carbon dioxide (CO2) from the combustion when mixing the ethanol and petrol in correct blend ratio for powering engine. The specific objective of the work is to investigate and optimize the process variables in the production of ethanol from cassava waste slurry.

2.0 Materials and Method

Cassava waste slurry (mixture of starch and water) was collected from a cassava processing plant located at National root crop research institute Umudike, Abia State during processing. A clean plastic container was used for sample collection.

The yeast cells used were fresh Sacchromyces cerevisiae (baker yeast) purchased from a local vendor in Umuahia, Abia State. Design Expert 7 software by Stat-Ease Inc., was used in implementing the optimization strategy. This software is a specialized statistical package for the design of experiments, which offers tools for response surface methods (RSM) with unique evaluation capabilities.

2.1 Experimental Set up

The experiment was setup in Chemical Engineering laboratory Michael Okpara University of Agriculture Umudike, Abia state in a two different process fermentation and distillation. The glass bioreactor, fabricated impeller attached to a motor, heating element with temperature controller, thermometer, beaker, condenser unit, pipette, round bottom flask, retort stand and heating mantle were assembled together before the experiment as shown in Figure 1. The bioreactor is a vendor packaged glass that can withstand the process temperature of between 25 – 70℃. It has six orifices with cork created to allow feeding of raw materials, ejection of product, insertion of instrument and integration of other glass wares accessories.

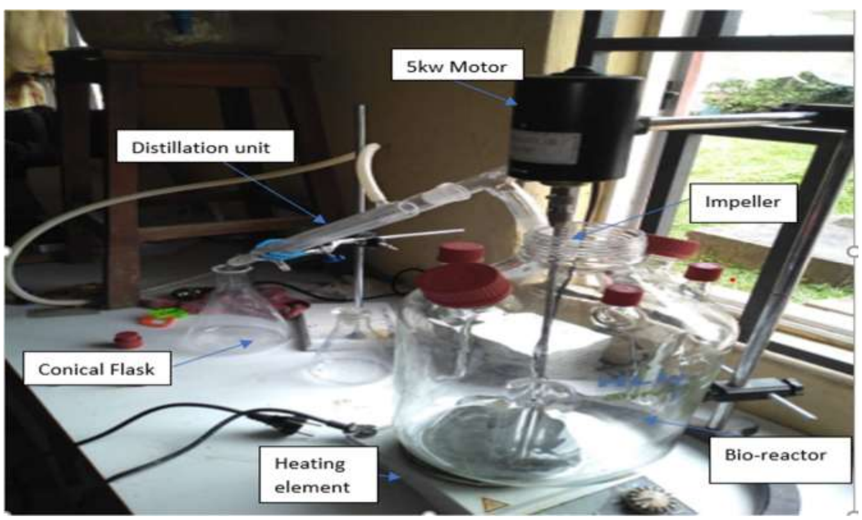

Figure 2.1: *Experimental set up for ethanol production*

A 5-kw motor attached with a fabricated spindle and impeller for stirring of the feedstock to achieve a homogenous mixture is mounted on top of the bio reactor. The bioreactor is placed on top of a heating element with temperature controller for temperature regulation. Thermometer was inserted inside the bioreactor and was firmly guarded with the cork. A glass pipe (condensing unit) functioning as a shell and tube heat exchanger was attached to the bioreactor for collection of mixture of ethanol and water into a stationed glass beaker. Two rubber pipes were connected to the two ends of the condenser; one for inlet cool water and the other for water outlet, and finally a glass beaker was stationed to the mouth of the condenser to collect the distillate.
The distillate in the glass beaker is then turned into a round bottom flask fixed with thermometer. This was

placed on top of a heating mantle attached with the condensing unit for pure ethanol distillation.

2.2 Experimental Procedure

2.2.1 Sample collection

Freshly discarded cassava waste slurry was obtained from a cassava processing plant located at National root crop research institute Umudike, Abia State using a plastic container. The yeast cells used in this study were fresh Saccharomyces cerevisiae (baker yeast) purchased from a local vendor in Umuahia, Abia State, Nigeria.

2.2.2 Development of inoculums

The inoculum was prepared by using Saccharomyces cerevisiae obtained from fresh palm wine and baker yeast equivalent to 10 g was added to 100 ml of 40℃ distilled water in a beaker to which 14 g of glucose was added to the inoculum and the yeast was allowed to grow. Thereafter, it was used in the cassava waste slurry.

2.2.3 Hydrolysis and fermentation

Cassava waste slurry (4litres) was measured in a transparent glass bioreactor and placed on an electric hot plate attached with temperature controller. Thereafter, 40ml of concentrated H_2SO_4 was added for pretreatment via the top of the bioreactor. A thermometer was firmly inserted to monitor the temperature of the solution. The motor was switched on to induce a vigorous stir within the reactor to achieve a homogenous mixture. The bioreactor was heated and at 80°C the solution began to gelatinize and foam. During this time the solution turned to pale orange color indicating a complete hydrolysis as shown in Figure 2.2. After heating, the sample was allowed to cool and settle, separating into three layers. The liquid layer was separated by decantation and filtration. The filtrate had a pH of 1.7 and was neutralized with 250ml of 2m NaOH solution to a pH of 4.8.

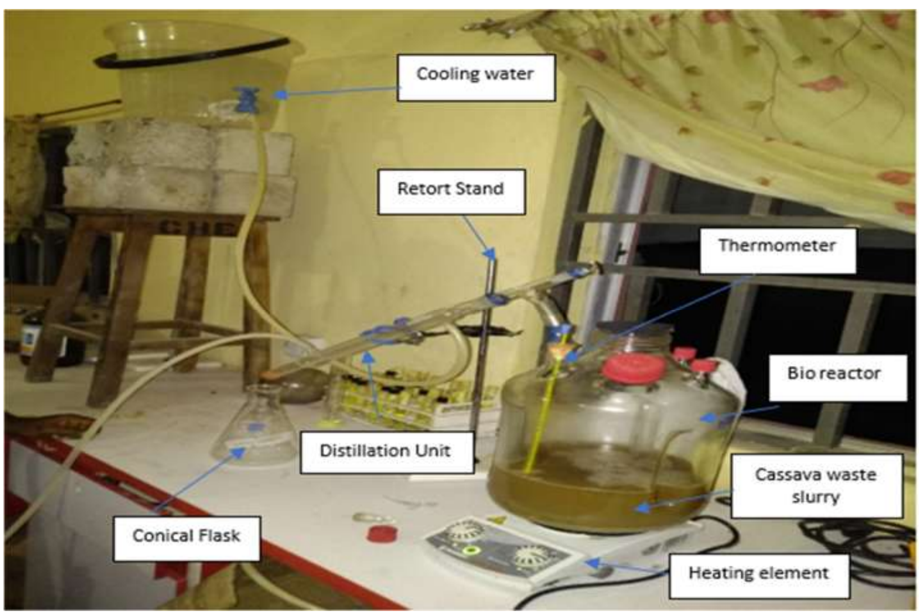

Figure 2.2: *Cassava waste water hydrolysis*

The solution was poured into different rinsed containers, following the addition of mixed 24.2g of yeast and 16g of glucose dissolved in 100ml of distilled water and allowed for 30mins to enable the yeast to grow. The containers were covered airtight and allowed fermentation to take place for 5 days at monitored temperature range of 25-40°C according the design of experiment. After 5 days, the fermented sample was transferred into a round bottom flask and placed on a heating mantle fixed to a distillation unit as shown in Figure 2.3. A conical flask was fixed to the other end of the distillation column and the ethanol was collected and analyzed for its concentration, yield, purity and specific gravity.

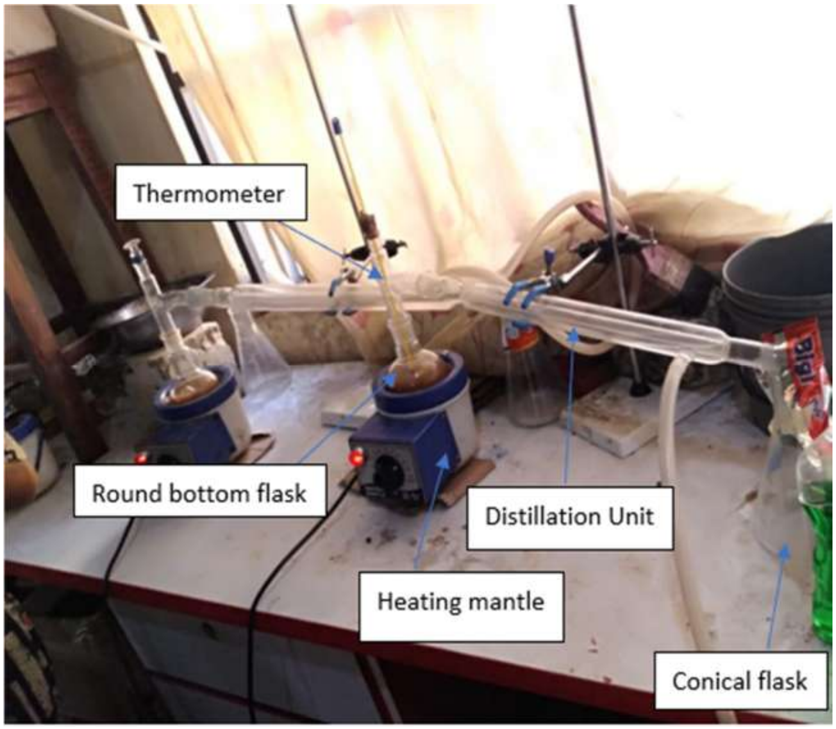

Figure 2.3: *Fermentation process*

The ethanol concentration was determined by the use of refractometer. Each sample of the ethanol was poured into the refractometer to read the refractive index. The refractometer takes refraction angles and correlates them to refractive index values that have been established. Using these values, the concentrations of the samples were obtained. This method was repeated for the number of experimental runs according to design of experiment.

2.2.4 Design of Experiment

The five (5) independent variable and their respective level selected for optimization of ethanol yield were as follows: process temperature (25, 32.5 and 40℃), PH (4, 5.25 and 6.5), sugar concentration (0.125, 0.23 and 0.35 mol/dm^3), time (0.5, 2.5 and 4.5 hours) and feed rate (100, 170 and 250 ml/hours). The levels were selected based on previous studies and the condition for ethanol experimental setup. For this work, the Design Expert software version 7.1.5, (Stat-ease, Inc, Minneapolis, USA) was used to design the experiment and analyze the data. The concept of randomization was utilized by the Design Expert software in generating the experimental design. This was done in order to minimize the effect of unexplained variability in the chosen responses (Montgomery, 2005). The effect of combination between the operation variables were studied with the use of a suitable experimental design that would allow the experimental results to be fitted to a polynomial response surface model. Central composite experimental design (CCD) matrix was chosen for this study to minimize the experimental runs and this involved 50 experimental runs. Central composite experimental design (CCD), with quadratic model (Box and Wilson, 1951) was employed to study the combined effect of five independent variables namely, temperature, PH, sugar concentration, time and feed rate of the fermenting medium. Optimization of the process parameters in ethanol fermentation using cassava waste slurry as medium was studied using response surface methodology (RSM).

3.0 Results and Discussion

3.1 Material analysis

The results of the proximate analysis carried out on cassava waste slurry is as shown in Table 3.1. The values of protein, ash content, total fat, moisture content, crude fiber, volatile matter, and fixed carbon of sample A, were 0.69, 0.48, 0.55, 1.48, 86.68, 2.60, 9.98 and 2.86 (%w/w) respectively. The proximate analysis results of the cassava waste slurry showed it contains more moisture, volatile matter, and fixed carbon.

Table 3.1: *Proximate Analysis of the cassava waste slurry*

Item	**% value**
Crude protein	0.69
Ash content	1.04
Total fat	0.55
Moisture content	86.83
Crude fiber	2.90
Volatile matter	9.98
Fixed carbon	6.82

3.2 Statistical Analysis

In this study, based on the employed independent variables, a CCD designed a set of experiments with 50 experiments for optimizing the production of ethanol. The results of the experimental runs were summarized in Table 3.2. The relationship between responses and independent variables were not linear, 2FI or cubic. Therefore, the experimental results were fitted to a second-order quadratic model using statistical technique. The final model equations in terms of actual experimental factors after elimination of the insignificant terms are shown in Table 3.3.

Table 3.2: *Experimental Result*

INDEPENDENT VARIABLES						RESPONSES		
Run	Temperature (^{0}C)	pH	Sugar Conc. mol/dm	Time (hrs)	Feed (mil)	Yield (%)	Purity (%)	Specific Gravity
1	40	6.5	0.35	0.5	100	8.5	6	0.248
2	25	6.5	0.125	4.5	100	6.4	2	0.266
3	25	4.0	0.35	4.5	250	14	4	0.275
4	40	4.0	0.125	0.5	100	9.2	6	0.294
5	25	4.0	0.125	0.5	100	11	10	0.365
6	40	6.5	0.35	0.5	250	18.8	8	0.288
7	25	4.0	0.35	0.5	250	13.2	4	0.255
8	25	4.0	0.125	0.5	250	17.6	8	0.305
9	40	6.5	0.35	4.5	100	9	10	0.201
10	40	4.0	0.35	0.5	100	14	6	0.301
11	40	4.0	0.35	4.5	250	23.2	4	0.322
12	32.5	5.25	0.23	2.5	175	13.1	4	0.257
13	32.5	5.25	0.23	2.5	175	10.3	2	0.186
14	40	6.5	0.35	4.5	250	12	8	0.216
15	32.5	5.25	0.23	2.5	175	8.6	2	0.194
16	40	4.0	0.125	0.5	250	26	6	0.243
17	32.5	2.27	0.23	2.5	175	4.6	8	0.184
18	32.5	5.25	0.23	2.5	175	11.4	4	0.242
19	25	6.5	0.125	0.5	250	17.2	6	0.266
20	25	4.0	0.125	4.5	100	12	2	0.174
21	40	6.5	0.125	4.5	250	19.2	2	0.234
22	25	6.5	0.35	4.5	100	8.4	12	0.149
23	25	4.0	0.35	0.5	100	9.2	8	0.164
24	32.5	5.25	0.23	7.26	175	12	10	0.332

25	40	4.0	0.125	4.5	250	26.8	6	0.138
26	25	6.5	0.35	0.5	100	5.6	8	0.358
27	32.5	5.25	0.23	2.5	338	0	0	0
28	25	6.5	0.35	4.5	250	13.6	4	0.212
29	14	5.25	0.23	2.5	175	9.7	8	0.236
30	32.5	5.25	0.23	2.5	353	21.2	4	0.139
31	25	6.5	0.125	4.5	250	15.6	4	0.185
32	40	6.5	0.125	0.5	100	4.3	8	0.279
33	40	4.0	0.35	0.5	250	10.8	6	0.193
34	32.5	5.25	0.24	2.5	175	11.4	10	0.216
35	40	6.5	0.13	0.5	250	14.8	6	0.206
36	39	5.25	0.24	2.5	175	6.4	12	0.317
37	32.5	5.25	0.50	2.5	175	6.06	4	0.292
38	32.5	5.25	0.23	2.5	175	8.11	10	0.307
39	32.5	5.25	0.23	2.5	175	8.9	12	0.321
40	32.5	5.25	0.23	2.5	175	7.3	8	0.295
41	40	6.5	0.125	4.5	100	7	6	0.268
42	40	4.0	0.125	4.5	100	6.4	4	0.225
43	25	6.5	0.35	0.5	250	14.8	10	0.283
44	40	4.0	0.35	4.5	100	9.2	6	0.217
45	25	4.0	0.125	4.5	250	24.4	14	0.359
46	32.5	5.25	0.23	2.5	175	9.14	8	0.231
47	32.5	5.25	0.03	2.5	175	12	10	0.248
48	25	6.5	0.125	0.5	100	8.5	6	0.263
49	25	4.0	0.35	4.5	100	9.2	6	0.239
50	32.5	5.25	0.23	2.25	175	12.9	10	0.295

The multiple regression analysis of the experimental data gave a second order polynomial equation depicting the interaction between the dependent variable (yield, purity and specifc gravity) and the coded values of the independent variables A, B, C, D, E (temperature, pH, sugar concentration, time and feed). This is shown in Table 3.3

Table 3.3: *Polynomial models for the response variables as a function of decoded operation variables for Ethanol production*

Response	2^{nd} Order polynomial equations
Yield	$+10.17 + 0.36A - 1.18B - 0.97C + 0.13D + 4.61E - 0.32A^2 + 2.85B^2 - 0.14C^2 + 0.90AE + 0.73BC - 0.33BD + 0.42DE$
Purity	$+5.23 - 0.2A + 0.1BE - 0.29A^2 - 0.061C^2 + 0.47D^2 - 0.24E^2 + 0.37AC + 0.12BE + 0.12CD - 0.25CE$
Specific Gravity	$+0.24 + 9.873E - 3.713E - 0.003A^2 + 0.013D^2 - 0.031E^2 - 0.003AD - 9.844E + 0.014CE + 6.094E - 0.03DE$

3.3 Analysis of Variance

The adequacy of the model for the yield of ethanol, purity of ethanol and specific gravity was justified through analysis of variance (ANOVA). The ANOVA was used to justify the suitability, statistical significance and fit of the model representing the responses yield, purity and specific gravity. The ANOVA for the quadratic models including the corresponding significant model terms, F-value, individual terms and lack of fit for the responses were shown in Table 3.4. The ANOVA test showed that the model F-value of ethanol yield, purity and specific gravity are 10.86, 2.65 and 3.71 respectively. Also, their model P-value in the model for yield and purity is <0.0001 and SG is 0.0007 implies that the model is highly significant, given that they are able to explain a 95% variability in all the responses considered and are useful for predicting the responses. However, p-value greater than 0.05 are considered to be insignificant which means that the changes in the values of the actual physical factor represented by that model term does not significantly affects the response under consideration (Lahijani *et al.,* 2013). Hence, the insignificant statistical terms could be ignored without damaging the model fitting. Moreover, lack of fit F-value of the yield, purity and specific gravity are 3.05, 0.640 and 0.55. This implies that lack of fit is not significant relative to the pure error.

The level of fit between the experimental data and the models was assessed using other statistical parameters such as coefficient of determination (R^2), standard deviation, coefficient of variation (CV)% etc as shown in Table 3.4. The quality of fitted quadratic model was expressed by the coefficient of determination (R^2), which represents the proportion of variability in a set of data that is accounted for a statistical model (Fermosso *et al.,* 2013). High R^2 value close to one is a desired value. As shown in Table 3.4 the R^2 value is high for all the models indicating very good fit between the experimental observations and model predictions. It also showed evidence the suitability of the quadratic model to interpret the experimental results.

Moreover, the adjusted R^2 values obtained were within reasonable agreement with the corresponding R^2 value further confirming the fit of the models (Fermosso *et al.,* 2013). The values of standard deviation were small compared to the mean and this shows that there was minimal dispersion of each individual observation about the mean (Montgomery,2005). This further confirmed the significant fit of the models. The coefficient of variation (C.V) is the standard deviation expressed as a percentage of the mean. It is a statistical parameter used to assess the reliability and repeatability of the experiments. Low C.V values like those presented in Table 3.4 indicates that the experimental runs are reliable and repeatable.

Table 3.4: *ANOVA evaluation of ethanol yield, purity and SG*

Response Variable	Yield	Purity	Specific Gravity
R^2	0.88	0.91	0.82
Adj-R^2	0.80	0.89	0.73
Pred.R^2	0.58	0.62	0.19
Std. Dev	2.77	1.2	0.40
Mean	12.76	6.04	2.4
CV (%)	21.69	21.01	16.18
Model F-value	10.86	2.65	3.71
Model P-value	<0.0001	<0.0001	0.0007
Lack of fit F-value	3.05	0.640	0.55
Lack of fit P-value	0.067	0.8010	0.87

3.4 Effect of process Parameters

Figure 4.1 shows the 3-dimensional plots with contour for yield of ethanol with respect to the combination effect of temperature and time of fermentation.

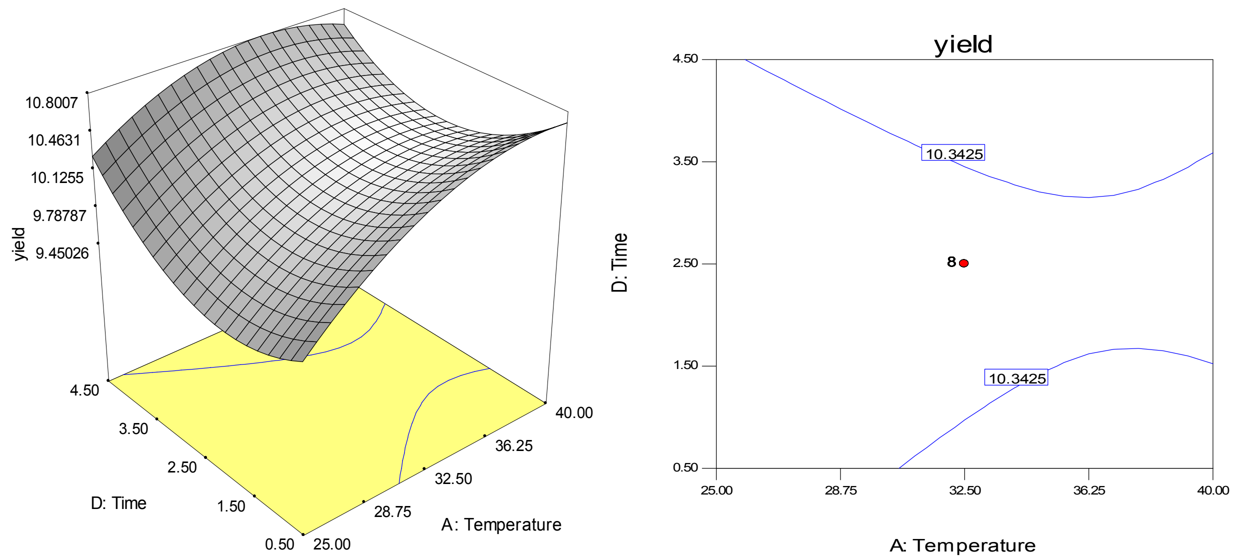

Figure 3.1: *Effect of reaction temperature and time on the yield of ethanol*

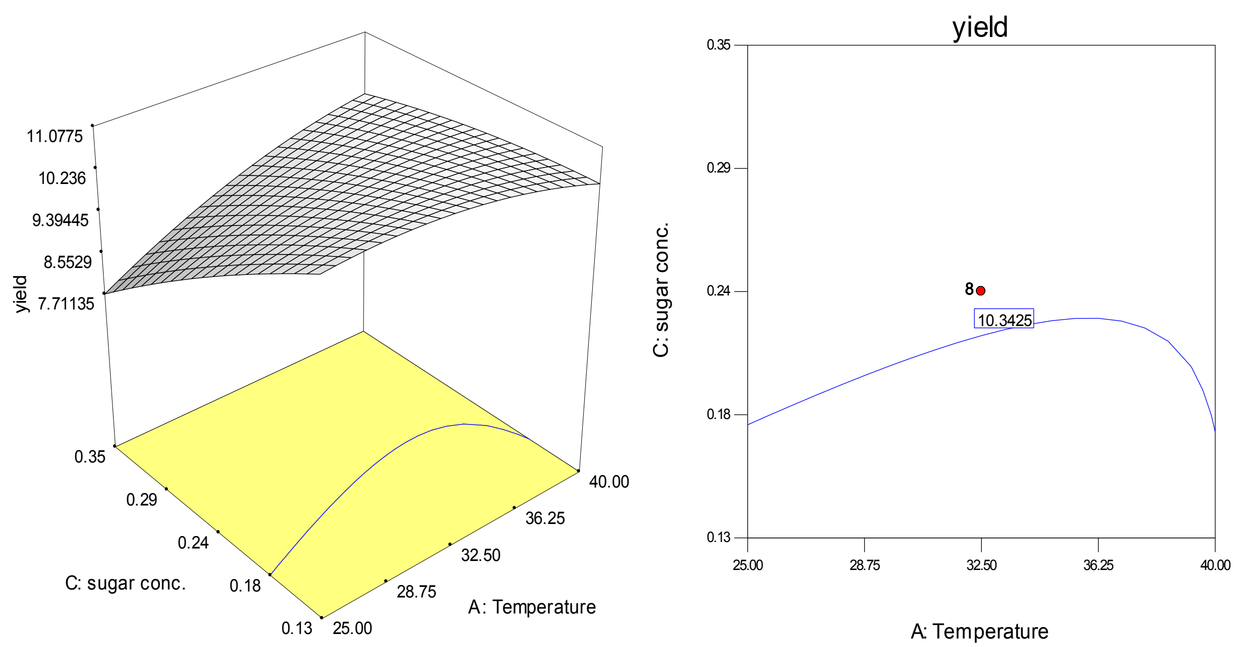

Figure 3.2: *Effect of temperature and sugar concentration on the yield of ethanol*

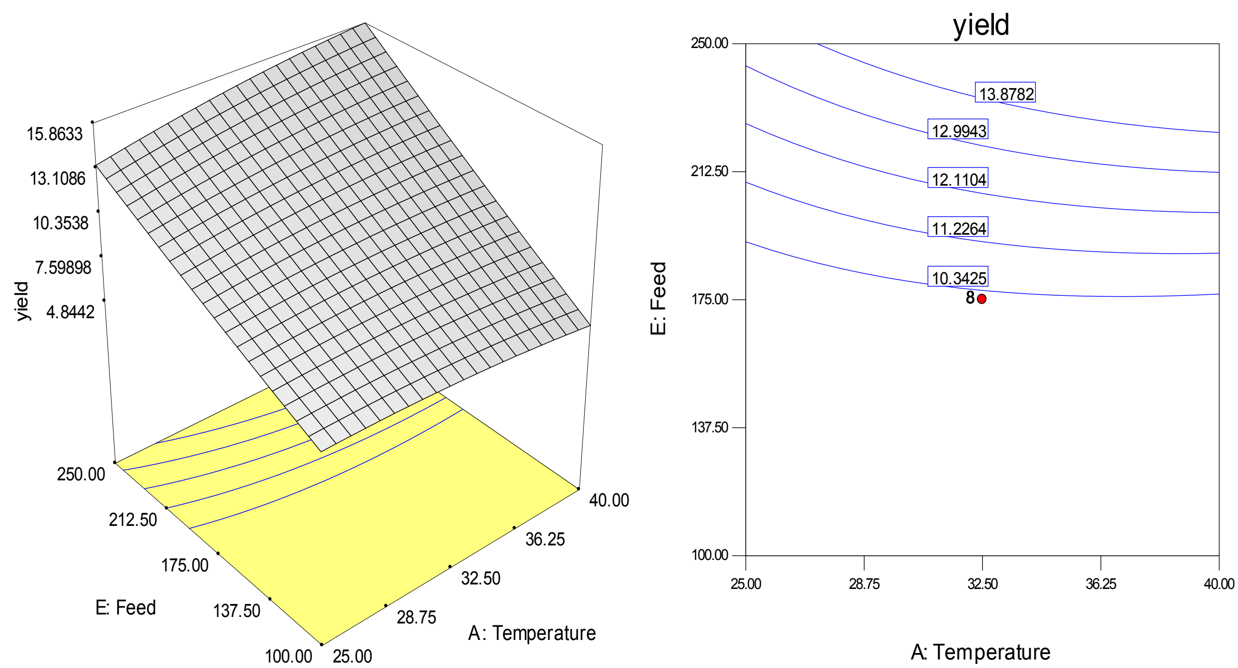

Figure 3.3: *Effect of reaction temperature and feed on the yield of ethanol*

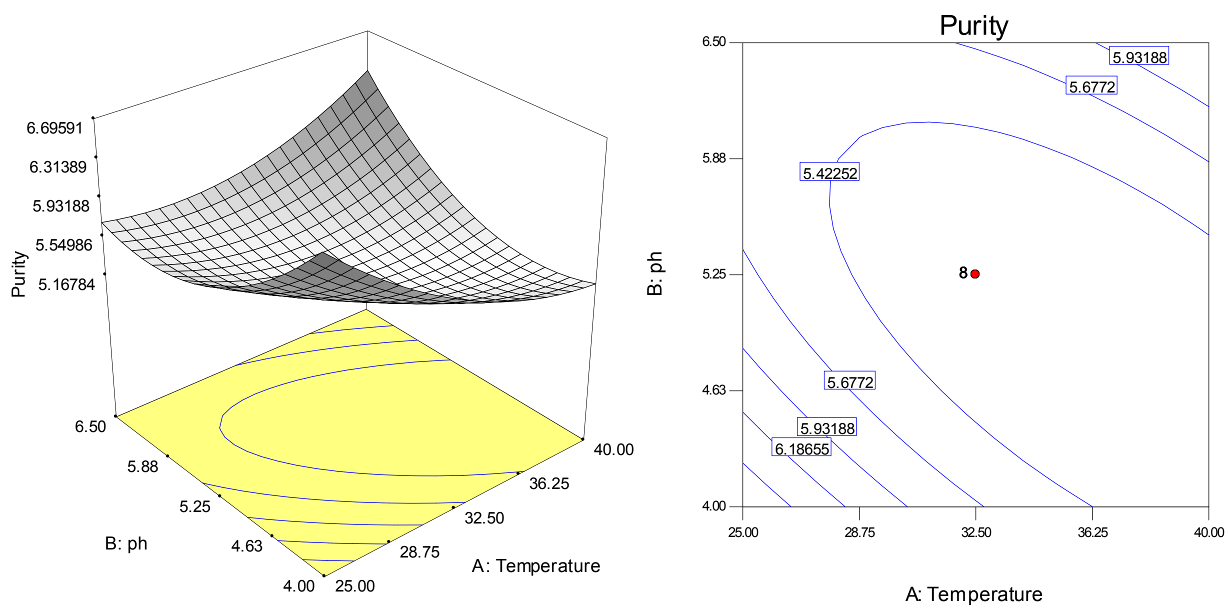

Figure 3.4: *Effect of reaction temperature and pH on the purity of ethanol*

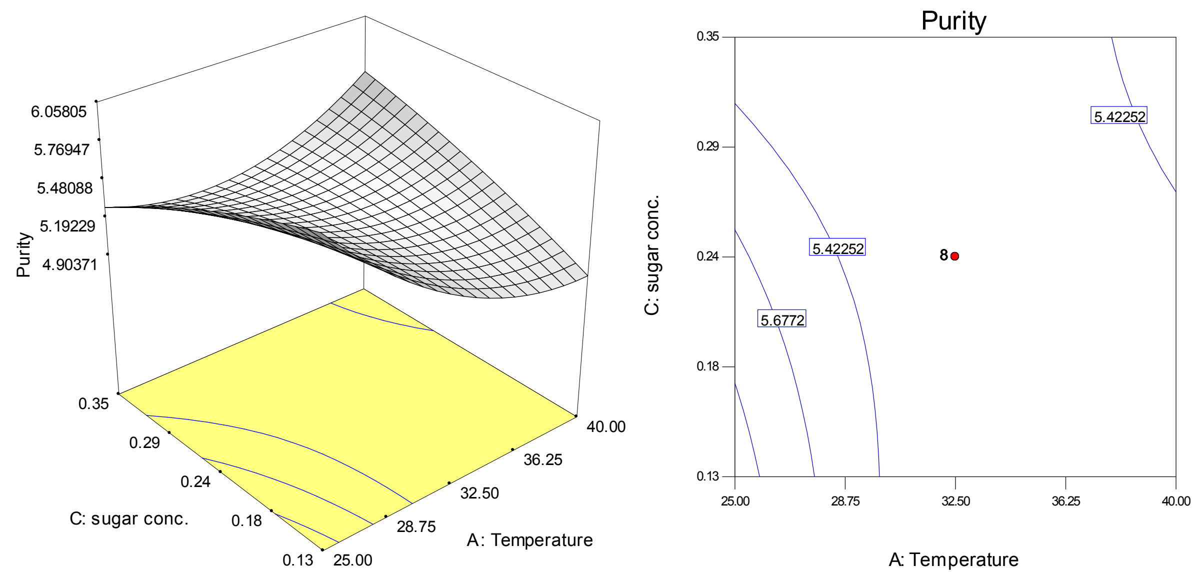

Figure 3.5: *Effect of reaction temperature and sugar concentration on the purity of ethanol*

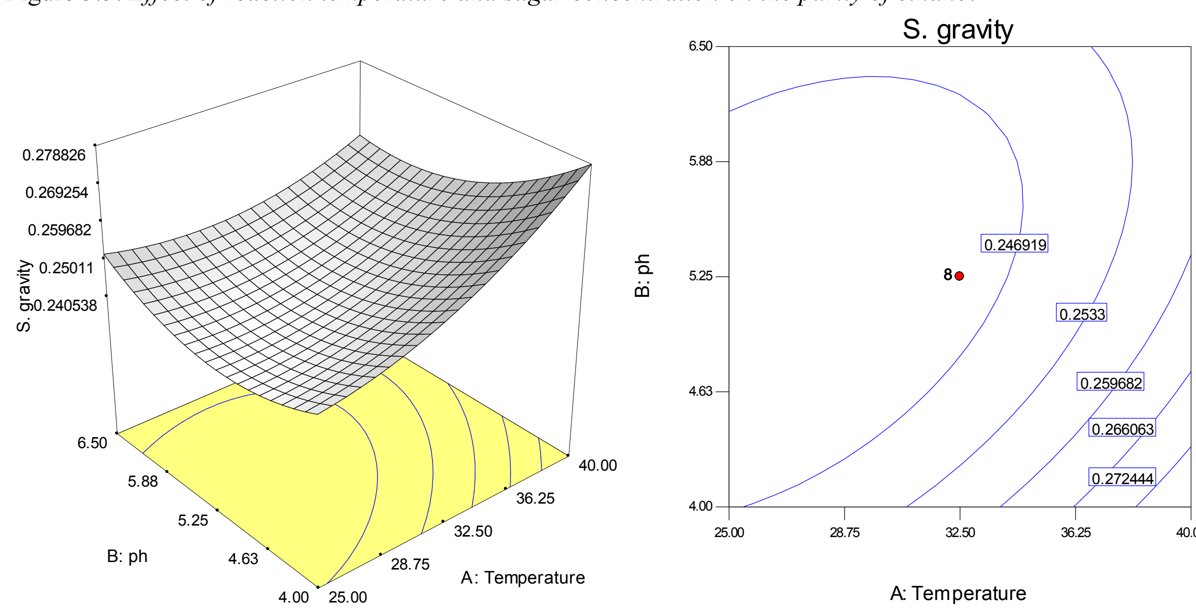

Figure 3.6: *Effect of reaction temperature and pH on the specific gravity of ethanol*

3.5 Optimization Of The Process

Determination of the optimum yield was based on numerical optimization using Design Expert software. The optimal yield of 10.54%, purity of 5.76% and specific gravity of 0.26 were obtained at reaction temperature of 30.06 ℃, pH of 5.40, sugar concentration of 0.30 ml/dm^3 and reaction time of 1.03 hours. Under these optimum conditions, the yield, purity and specific gravity were obtained at desirability of 1.000%.

4.0 Conclusion

This study was carried out in order to produce ethanol from waste generated from cassava processing. The waste generated from cassava was characterized using ultimate and proximate analysis. The process parameters for the synthesis of the ethanol was designed with Central Composite Design (CCD) and the effect of the process variables (temperature, pH, sugar concentration, time etc.) on the ethanol yield, purity and specific gravity were studied. The process parameters were optimized in order to obtain the optimal yield, purity and specific gravity. The proximate analysis results of the cassava waste slurry showed that it contains more moisture, volatile matter and fixed carbon. The experimental result showed that the maximum ethanol yield of 26% was obtained at a temperature of 40 ^{0}C, pH of 4, sugar concentration of 0.125 ml/dm^3, production time of 0.5hrs and feed value of 250 mil/hour. The statistical analysis of the yield, purity and specific gravity showed correlation coefficient of 0.88, 0.91 and 0.82 respectively.

The effect of the process parameters showed that increase in the reaction temperature, feed, time and pH increases the yield of ethanol while increase in the sugar concentration decreases the ethanol yield. Furthermore, increase in the reaction temperature, sugar concentration, time and pH decrease the purity of the ethanol while increase in the feed results to increase in the purity of the ethanol. Additionally, increase in the reaction temperature, sugar concentration and feed increase the specific gravity of the ethanol while increase in the pH and reaction time decreases the specific gravity of the ethanol.

The optimization result showed that the optimal yield of 10.54%, purity of 5.76% and specific gravity of 0.26 were obtained at reaction temperature of 30.06℃, pH of 5.40, sugar concentration of 0.30ml/dm^3 and reaction time of 1.03 hrs.

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