Modeling of a Still Reactor Banana Peels Fermentation: Waste to Energy Approach

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Abstract Limited information on optimal biofuel production conditions leads to non-economical and inefficient process hence uncompetitive low grade biofuels. Fermentation process optimization is very crucial especially while using relatively low fermentable sugars substrates. In this research, banana peels derived from *Ngombe* cultivar were dried, ground into fine powder to pass through a 1 mm screen, and then hydrolyzed using 60% concentrated H_2SO_4 at 50°C. Bioethanol was produced by anaerobic fermentation of the hydrolysate using *Saccharomyces cerevisiae*. Erlenmeyer Flasks fitted with non-return air valves were used as laboratory scale still reactors. Fermentation systems were subjected to various conditions based on half factorial Central Composite Rotatable Design (CCRD). Total Reducing Sugars (TRS) concentrations and bioethanol yield analyses were done by Dubois and Gas Chromatography methods respectively. Optimum bioethanol yield of 13.09 ml/L was obtained at 180 g/L substrate concentration, 35°C fermentation temperature, 5.5 initial medium pH, 2 g/L yeast concentration, and 120 hours incubation corresponding to a TRS degradation of 30.30 g/L. Lowest yield of 1.44 ml/L was obtained at 84.86 g/L substrate concentration, 35°C fermentation temperature, 5.5 initial medium pH, 2 g/L yeast concentration, and 120 hours incubation corresponding to a TRS degradation of 2.85 g/L. Maximum bioethanol yield realized from these wastes manifested viable concentrations which could further be distilled and dried to be used as an energy resource. The mathematical model developed also posed as a predictive tool on bioethanol yield while using banana peels and similar wastes in energy resource generation.

Keywords Anaerobic, banana peels, bioethanol, Central Composite Rotatable Design (CCRD).

1. Introduction

Ethanol can directly be used as a fuel or mixed with gasoline to act as an octane enhancer [1]. It is a proven source of energy as it burns to produce heat energy alongside other products. Ethanol derived from biological fermentation of carbonaceous feedstock e.g. banana peels, molasses, sorghum amongst others is referred to as bioethanol. Ethanol is less toxic as compared to fossil fuels and is biodegradable.

In Kenya, Horticultural Crops Development Authority (HCDA) cited 15 different varieties of banana [1] which are grown in different parts of the country. Banana production is widely spread across Kenyan counties. Banana withstands short flooding periods as long as there is adequate soil aeration [1].

In Kenya, banana production (Fig. 1) has been rising in the past years. Productions of about 2.0 M and 1.7 M metric tons for 40% under Tissue Culture (TC) banana and 25% under TC respectively are predicted for the year 2016 assuming total land coverage of 90,580 ha [2].

It has been established through research that about 10% of Musa Spp. is comprised of wastes including stems, skins or peels, and leaves [3]. This enormous quantity of banana wastes can be converted to bioethanol to assist in meeting the energy demand especially in the automotive industry. Banana peels are common food wastes usually discarded because people feel they lack economic value [3]. They have relatively large sugar content which can be harnessed into bioethanol production rather than being discarded as wastes. They have enormous potential in bioethanol industry and can be used as a cheaper source of alternative fuel. Banana plant parts including stems, fruits, pulps, and peelings have been used as bioethanol production feed-stocks [5]-[6].
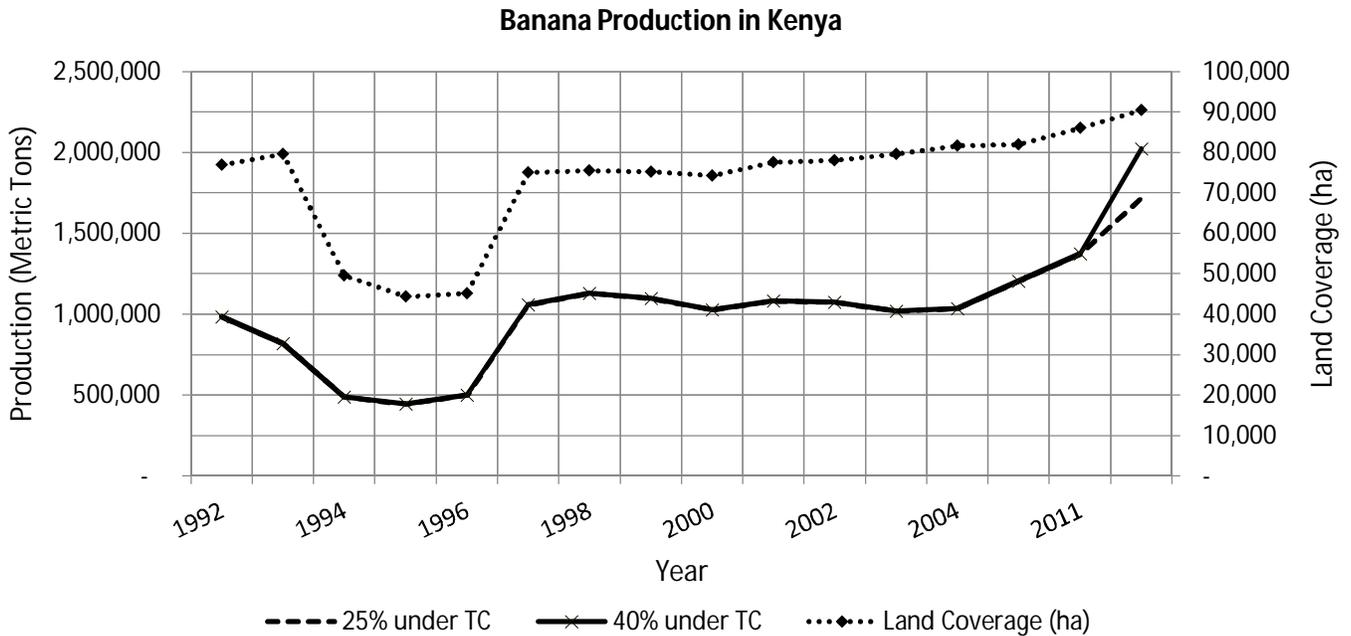
Banana Production in Kenya

Fig. 1: Banana Production and Area under Banana Plantation in Kenya [2].

Quality and efficiency are some of the major concerns in every manufacturing establishment. Possibility of achieving high grade products with minimum resources can translate into widening of profit margins. Efficient operations can reduce value addition lead time and wastes if appropriate measures are taken. In bioethanol production, knowledge of optimal process parameters, particularly those affecting fermentation can greatly enhance interest in the process. This knowledge can enhance interest in bioethanol production from wastes thus leading to enhanced energy supply for development.

Response Surface Methodology (RSM) comprises of statistical and mathematical techniques used to generate models and consequently analyze problems. The main concept is determining optimal responses [3]. Design of Experiments (DoE) is applied in the organization experiments in RSM.

Product quality in a manufacturing establishment can be improved by integrating DoE in the early stages of developments cycle [4]. Central Composite Design (CCD) developed by Box and Wilson in 1951 is one of the techniques used in optimization of experimental conditions. CCD combines 2^k full factorial or 2^{k-p} fractional experimental runs, 2^k star or axial point experimental runs on each k axis of distance $\pm\propto$ away from the central point and at least one center point experimental run, where k, p, and α are number of factors in the optimization experiment, number with which the

runs are fractioned, and axial point distance respectively.

In this research, optimal experimental conditions involving *Ngombe* peels fermentation were to be determined through both the experimental and statistical approaches. A model was generated for application to similar processes.

2. Materials and methods

Research station was the Public Health Laboratory of Moi University, Eldoret-Kenya.

2.1. Substrate

Banana peels were used as substrates throughout the study. Fruits of banana (*Ngombe* cultivar) purchased from Kenya Agricultural Research Institute (KARI), Kisii Branch were washed with tap water and then peeled. The peels were cut into 2 to 3 cm sizes, sundried for 10 days, and further oven dried at 60°C for 24 hours. The dried *ngombe* peels were finally milled to pass through a 1mm screen.

2.2. Micro-Organism

Saccharomyces cerevisiae was used throughout the study. Stock yeast culture was maintained on 0.3 g/L NH_4Cl, 0.1 g/L KH_2PO_4, 1.0 g/L $MgSO_4.7H_2O$, 0.06 g/L K_2HPO_4, 5 g/L yeast extract, 10 g/L glucose, 10 g/L peptone, and 20 g/L agar.

Inoculation medium containing 0.5 g/L KH_2PO_4, 1.0 g/L $MgSO_4.7H_2O$, 5 g/L yeast extract, 0.4 g/L NH_4Cl, and

0.02 g/L glucose was adjusted to pH 5.5 using 1 M KOH/H$_2$SO$_4$. The medium was then autoclaved at 120°C and 1bar for 10 min, cooled to 20°C and thereafter inoculated with 10% v/v yeast colony. The inoculum was incubated at 35°C for 24 hours while being shaken at approximately 120rpm.

2.3. Substrate Hydrolysis

576 g peels powder was impregnated with 1152 ml 60% H$_2$SO$_4$, maintained at 50°C in a water bath while being agitated at 200rpm for 60min.

2.4. Hydrolysates detoxification

Detoxification was done by over-liming. Powdered Ca(OH)$_2$ was added gradually while agitating at 200rpm until a pH slightly above 8.00 was attained.

2.5. Adjustment of Fermentation Conditions

Factor ranges shown on Table 1 below were chosen based on past related research activities. They covered values which had worked in nearly similar industrial procedures.

Table 1: Factors description

Factor	Axial (-α)	Min (-1)	Center (0)	Max (+1)	Axial (+α)
X$_1$ (g/l)	84.86	140.00	180.00	220.00	275.14
X$_2$ (^{o}C)	23	30	35	40	47
X$_3$	3.12	4.50	5.50	6.50	7.88
X$_4$ (g/l)	0.81	1.50	2.00	2.50	3.19
X$_5$ (hrs)	62.92	96.00	120.00	144.00	177.08

X$_1$: Substrate Concentration, X$_2$: Fermentation Temperature, X$_3$: Initial Medium pH, X$_4$: Yeast Concentration, and X$_5$: Incubation Period

Substrate concentrations, X$_1$ were prepared in bulk amounts to reduce associated random errors. All the five bulk samples: 84.86, 140.00, 180.00, 220.00, and 275.14 g/L were filtered through Whatman No. 1 filter paper using a vacuum filter and then stored at 4°C for further fermentation procedures. Media pH was adjusted using 1M KOH/H$_2$SO$_4$.

2.6. Fermentation

100 ml media containing specific substrate concentrations (Table I), nutrients (1.6 g/L NH$_4$Cl, 0.1 g/L KH$_2$PO$_4$, 4.1 g/L MgSO$_4$.7H$_2$O, 0.2 g/L K$_2$HPO$_4$, 3 g/L yeast extract, 3.0 g/L NH$_4$C$_2$H$_3$O$_2$, 10 mg/L C$_2$H$_7$NO$_2$), and 0.1% v/v mineral solution (15 g/L MnSO$_4$.H$_2$O, 25 g/L FeSO$_4$.7H$_2$O, 12 g/L Na$_2$SO$_4$, 20 g/L CaCl$_2$.6H$_2$O, and 68 g/L MgSO$_4$.7H$_2$O) were usually autoclaved, cooled to 20°C, and then inoculated with 4.1%, 7.5%, 10.0%, 12.5% and 16.0% v/v yeast culture based on yeast concentration level, coked with a non-return air valve and then incubated for specific periods based on X$_4$.

2.7. Analyses

2.7.1. Moisture content

Substrate moisture content was analyzed according to the method used by Arumugam and Manikandan in 2011. 10g of banana peels powder was dried to a constant weight at 105°C and then the dry weight was calculated as shown in equation (1) below.

$$M_D = \frac{W - D}{D} x100 \qquad (1)$$

2.7.2. Ash content

Ash content was analyzed according to the United States Department of Energy (USDOE) Standard Biomass Laboratory Analytical Procedures. 10g of banana peels was weighed into a crucible and heated at 550°C for 24 hours. The residue was then weighed and the percentage ash content calculated as shown in equation (2) below.

$$Ash(\%) = \frac{w_f}{w} x100 \qquad (2)$$

2.7.3. Total Reducing Sugars (TRS)

TRS concentrations were analyzed by the Dubois method. 2 ml of each diluted supernatant was pipetted into various test-tubes. 0.05 ml of 80% phenol solution was then added to each of the nine test-tubes containing the hydrolysate supernatant. 5 ml of 95.5% Sulphuric acid was then dispensed into the mixture at the middle of the test tubes using a burette to facilitate rapid mixing and reaction rate. The mixtures were allowed to rest for 10 minutes and thereafter shaken. They were then put in 30°C water bath for 20 minutes.

Analyses for TRS were done using Jenway 6051 Colorimeter (which had been allowed a 15 minutes warm up time) at 490 nm wavelength.

2.7.4. Bioethanol Yield

A GC (Shimadzu 2010) was used in the quantitative analysis of ethanol in all the fermentation media. GC settings and characteristic features (Table 2) were selected so as to enable ethanol separation from the injected supernatant.

Table 2: GC analysis description

GC	Shimadzu GC 2010
Injector	Temperature: 150°C Split ratio: 5.1 Total Flow: 81.2 ml/min Purge flow: 3 ml/min
Column	ZB-Wax; Length 30m; Inner Diameter 0.32mm; Film thickness 0.25µm
Packing	Polyethylene glycol
Column	2 min initial hold time, 40°C to 220°C at 20°C/min, 4 min final hold time (Temperature program) Column flow: 12.82 ml/min Linear velocity: 120.6 cm/sec
Nitrogen (Carrier gas)	Temperature: 150oC Pressure: 227.9kPa Total Flow: 81.2 ml/min Purge flow: 3 ml/min
Hydrogen (Detector)	Temperature: 280oC Flow: 80 ml/min Make up flow: 20 ml/min
Air	Flow: 400 ml/min
Detector	Flame Ionization Detector (FID)
Software	GC Solution

4.1. Statistical Analysis

Response Surface Methodology (RSM) was employed in determining optimal responses. The predicted response was given by the following second order polynomial.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum_{i<j}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad (3)$$

Where:

   Y=predicated response
   β=Regression coefficient
   i, j= linear, quadratic co-efficient respectively
   k=number of independent factors
   ε=random error.

Half factorial CCRD of 31 runs was designed on MATLAB 7.11.0.584 (R2010b). Co-efficient of Correlation (R^2), Fischer (F-test), and student (t-test) were used to test the statistical significance of the fitted data.

3. Results and discussion

Non-edible parts constituted 43.42% w/w of fresh banana bunch while wet peels constituted of 40.28% w/w of the fruits. This big percentage of food waste always poses a serious wastage handling and disposal problem. Ground peels powder was 4.55, 4.80, and 11.92% w/w of the whole bunch, fruits, and wet peels respectively.

This implies that about 88.08% w/w of banana peels comprised of water which closely compares with a range of 82.47- 86.21% w/w reported by Nuttiya and Jirasak [7] and 78.9% w/w given by Srishail [8].

Table 3: Banana peels characterization

Parameters	Quantity
Moisture (% Dry Weight)	8.02
Ash content (% w/w)	7.85
TRS (% w/w)	36.21

Dry feed-stocks are very convenient to handle and are not vulnerable to microbial degradations (Table 3). These dry weights were slightly higher than 5.00% w/w found by collecting banana peels from a market, air drying for a few days, and two days oven drying at 60°C [9]. Similarly, they were slightly higher than 6.70% w/w found by Srishail in analyzing banana peels nutritional composition [8].

Ash contains minerals affecting yeast metabolism. These wastes are therefore minerals rich for yeast fermentation. The ash content closely compares with 8.50% w/w found by Srishail in analyzing banana peels nutritional composition [8].

Acidic hydrolysis raised TRS composition from 15.52% to 36.21% w/w. This high fermentable sugars composition makes these wastes a suitable substrate for bioethanol production. TRS concentration with 0% H_2SO_4 hydrolysis was slightly higher than 15.2% w/w achieved by distilled water dilution and 30 minutes boiling of banana peels [9].

Optimum responses were shown by the smallest ellipses on contour plots. Experiments showed the highest actual bioethanol yield (un-distilled broth) of 13.09 ml/L corresponding to a predicted bioethanol yield (un-distilled broth) of 12.87 ml/L at 180 g/L substrate concentration, 35°C fermentation temperature, 5.5 initial medium pH, 2 g/L yeast concentration and 120 hours incubation period (Table 4). Similarly, they exhibited the lowest bioethanol yield (un-distilled broth) of 2.32 ml/L corresponding to a predicted bioethanol yield (un-distilled broth) of 0.86 ml/L at 84.86 g/L substrate concentration, 35°C fermentation temperature, 5.5 initial medium pH, 2 g/L yeast concentration and 120 hours incubation.

Table 4: Experimental design table, actual and predicted responses

Run	X_1 (g/L)	X_2 (^oC)	X_3 (pH)	X_4 (g/L)	X_5 (hours)	Actual TRS (g/L)	Actual EtOH (ml/L)	Predicted TRS (g/L)	Predicted EtOH (ml/L)
1.	0	0	0	0	0	29.45	12.92	29.40	12.87
2.	0	0	0	0	0	28.61	12.76	29.40	12.87
3.	0	0	0	0	0	30.30	13.09	29.40	12.87
4.	0	0	0	0	0	28.61	12.8	29.40	12.87
5.	0	0	0	0	0	29.88	12.71	29.40	12.87
6.	1	-1	-1	-1	-1	12.56	5.65	12.65	5.67
7.	-1	1	-1	-1	-1	12.98	5.82	12.24	5.48
8.	-1	-1	1	-1	-1	7.07	3.17	6.83	3.04
9.	1	1	1	-1	-1	19.74	8.82	20.90	9.30
10.	-1	-1	-1	1	-1	8.34	3.78	7.03	3.21
11.	1	1	-1	1	-1	15.52	6.82	15.60	6.86
12.	1	-1	1	1	-1	18.9	8.48	19.48	8.73
13.	-1	1	1	1	-1	17.63	7.87	17.39	7.76
14.	-1	-1	-1	-1	1	13.83	6.15	12.82	5.68
15.	1	1	-1	-1	1	15.52	6.99	15.91	7.14
16.	1	-1	1	-1	1	19.32	8.64	20.20	8.99
17.	-1	1	1	-1	1	20.17	9.19	20.23	9.19
18.	1	-1	-1	1	1	23.12	10.3	22.93	10.21
19.	-1	1	-1	1	1	21.85	9.74	20.84	9.30
20.	-1	-1	1	1	1	17.21	7.63	16.68	7.39
21.	1	1	1	1	1	26.92	12.01	27.80	12.39
22.	-2.3784	0	0	0	0	2.85	1.44	4.82	2.32
23.	2.3784	0	0	0	0	18.9	8.49	17.13	7.74
24.	0	-2.3784	0	0	0	10.03	4.46	10.61	4.74
25.	0	2.3784	0	0	0	20.58	9.21	20.20	9.05
26.	0	0	-2.3784	0	0	9.61	4.28	11.03	4.91
27.	0	0	2.3784	0	0	21.01	9.35	19.80	8.85
28.	0	0	0	-2.3784	0	15.52	6.89	15.13	6.77
29.	0	0	0	2.3784	0	22.27	9.91	22.86	10.15
30.	0	0	0	0	-2.3784	8.34	3.76	8.46	3.82
31.	0	0	0	0	2.3784	21.85	9.78	21.93	9.84

This optimum ethanol yield was higher than 2.7 ml/L found by fermenting hydrolyzed red banana peel in 10% substrate concentration [5], 11.41 ml/L gotten from banana peels fermentation using mutant strain of *Saccharomyces cerevisiae* [6], 10.14 ml/L achieved through Simultaneous Saccharification and Fermentation (SSF) process involving co-culture of *Saccharomyces Cerevisiae* and *Candida tropicalis* [7], and 13.00 ml/L gotten through Separate Hydrolysis and Fermentation (SHF) process involving co-culture of *Saccharomyces Cerevisiae* and *Candida tropicalis* [7].

However, higher ethanol production of 39.29 ml/L has been achieved by fermenting banana peels within 72 hours incubation period [9], 35.74 ml/L from hydrothermally pre-treated banana peeling by optimizing Simultaneous Saccharification and Fermentation (SSF) processes [10], and 19.00 ml/L from banana peels fermentation with 96 hours incubation [11].

These differences could be majorly attributed to fermentation techniques as well as the microorganism purity, culturing procedures, and other factors affecting fermentation.

Table 5: ANOVA Table ($\alpha=0.05$, $R^2=0.9874$, and Adjusted $R^2=0.9622$)

Source	d.o.f.	Sum Sq.	Mean Sq.	F.	$F_{crt.}$	Comment
Model	20	296.9101	14.8455	39.1485	2.77	Significant
X_1	1	46.6807	46.6807	123.0999	4.96	Significant
X_2	1	29.5292	29.5292	77.8703	4.96	Significant
X_3	1	24.6473	24.6473	64.9964	4.96	Significant
X_4	1	18.0998	18.0998	47.7303	4.96	Significant
X_5	1	57.5358	57.5358	151.7254	4.96	Significant
X_1^2	1	6.6564	6.6564	17.5533	4.96	Significant
$X_1 X_2$	1	2.1170	2.1170	5.5827	4.96	Significant
$X_1 X_3$	1	0.4970	0.4970	1.3106	4.96	Insignificant
$X_1 X_4$	1	0.9506	0.9506	2.5068	4.96	Insignificant
$X_1 X_5$	1	2.6244	2.6244	6.9207	4.96	Significant
X_2^2	1	0.0578	0.0578	0.1523	4.96	Insignificant
$X_2 X_3$	1	0.5780	0.5780	1.5242	4.96	Insignificant
$X_2 X_4$	1	0.0010	0.0010	0.0026	4.96	Insignificant
$X_2 X_5$	1	0.2450	0.2450	0.6461	4.96	Insignificant
X_3^2	1	1.7030	1.7030	4.4909	4.96	Significant
$X_3 X_4$	1	813.1891	813.1891	2144.4295	4.96	Significant
$X_3 X_5$	1	1018.9211	1018.9211	2686.9574	4.96	Significant
X_4^2	1	1016.5982	1016.5982	2680.8317	4.96	Significant
$X_4 X_5$	1	1208.9117	1208.9117	3187.9742	4.96	Significant
X_5^2	1	1011.3812	1011.3812	2667.0742	4.96	Significant
Error	10	3.7921	0.3792			
Total	30	300.7022				

From the ANOVA (Table 5), high co-efficient of determination, R^2 value of 0.9874 with an adjusted R^2 of 0.9622 shows that the above 2nd order polynomial regression (3) excellently evaluates the experimental data involving bioethanol yield from banana peels hydrolysate derived from the *Ngombe* cultivar.

Bioethanol yield model F-value of 39.1485, higher than the critical value of 2.77 clearly shows that this model is significant to evaluate experimental data involving bioethanol yield from banana peels hydrolysate. The probability of bioethanol yield responses occurring due to noise was less than 5%. This further asserts that this model is significant.

The minimum p values of X_1 (substrate concentration), X_2 (fermentation temperature), X_3 (initial medium pH), X_4 (yeast concentration) and X_5 (hours incubation) in the ANOVA table (Table 5) above shows significant positive contribution of these five independent factors to the bioethanol yield from banana peels hydrolysate derived from the *Ngombe* cultivar. All the main effects were therefore significant in this experiment.

From the ANOVA, response surfaces and the contours (Fig. 2 to 6) above, the interactions between X_1 (substrate concentration) and X_2 (fermentation temperature), X_1 (substrate concentration) and X_5 (hours incubation), X_3 (initial medium pH) and X_4 (yeast concentration), X_3 (initial medium pH) and X_5 (hours incubation), and X_4 (yeast concentration) and X_5 (hours incubation) significantly contributed to bioethanol yield from banana peels hydrolysate. However, the interactions between X_1 (substrate concentration) and X_3 (initial medium pH), X_1 (substrate concentration) and X_4 (yeast concentration), X_2 (fermentation temperature) and X_3 (initial medium pH), X_2 (fermentation temperature) and X_4 (yeast concentration), and X_2 (fermentation temperature) and X_5 (hours incubation) made insignificant contribution to this yield. All the squared interactions of X_1 (substrate concentration), X_2 (fermentation temperature), X_3 (initial medium pH), X_4 (yeast concentration), and X_5 (hours incubation) significantly contributed to bioethanol yield from banana peels hydrolysate.

Table 6: t-TEST (t_{crt}=2.228, *Slightly Insignificant)

Source	Co-eff Estimate	Std. Error	t.	Comment
Intercept	12.8724	0.2728	47.1876	Significant
X_1	1.1396	0.1178	9.6720	Significant
X_2	0.9064	0.1178	7.6926	Significant
X_3	0.8281	0.1178	7.0280	Significant
X_4	0.7096	0.1178	6.0226	Significant
X_5	1.2652	0.1178	10.7379	Significant
X_1^2	-1.3868	0.0891	15.5596	Significant
$X_1 X_2$	-0.6450	0.1540	4.1897	Significant
$X_1 X_3$	0.3638	0.1540	2.3628	Significant
$X_1 X_4$	0.1763	0.1540	1.1449	Insignificant
$X_1 X_5$	-0.2438	0.1540	1.5833	Insignificant
X_2^2	-1.0563	0.0891	11.8507	Significant
$X_2 X_3$	0.4050	0.1540	2.6307	Significant
$X_2 X_4$	-0.0600	0.1540	0.3897	Insignificant
$X_2 X_5$	-0.1900	0.1540	1.2342	Insignificant
X_3^2	-1.0598	0.0891	11.8904	Significant
$X_3 X_4$	0.0088	0.1540	0.0568	Insignificant
$X_3 X_5$	-0.1238	0.1540	0.8038	Insignificant
X_4^2	-0.7796	0.0891	8.7467	Significant
$X_4 X_5$	0.3263	0.1540	2.1192	*Insignificant
X_5^2	-1.0677	0.0891	11.9796	Significant

A two tailed student (t) test at 95% confidence level (Table 6) was further carried to evaluate significance of various regression co-efficient associated with the above regression polynomials.

Regression co-efficient associated with interaction terms X_1 (substrate concentration) and X_4 (yeast concentration), X_1 (substrate concentration) and X_5 (hours incubation), X_2 (fermentation temperature) and X_4 (yeast concentration), X_3 (initial medium pH) and X_4 (yeast concentration), X_3 (initial medium pH) and X_5 (hours incubation), and X_4 (yeast concentration) and X_5 (hours incubation) were insignificant and therefore collated with other errors as ε_{EtOH} in the bioethanol yield model.

The second order regression polynomial (4) below predicts bioethanol yield from banana peels fermentation.

$$Y_{EtOH} = 12.8724 + 1.11396X_1 + 0.9064X_2 + 0.8261X_3 + 0.7096X_4 + 1.2652X_5$$
$$-1.3868X_1^2 - 0.6450X_1X_2 + 0.3638X_1X_3 - 1.0563X_2^2 + 0.4050X_2X_3$$
$$-1.0598X_3^2 - 0.7796X_4^2 + 0.3263X_4X_5 - 1.0677X_5^2 + \varepsilon_{EtOH} \quad (4)$$

In these experiments, bioethanol concentration increased with increasing substrate concentrations up to an optimum level of 180 grams of banana peels litre of the fermentation broth. At high substrate sugars, yeast cells might have overcome osmotic stresses attributed to

increased bioethanol concentrations in the bioreactor [12].
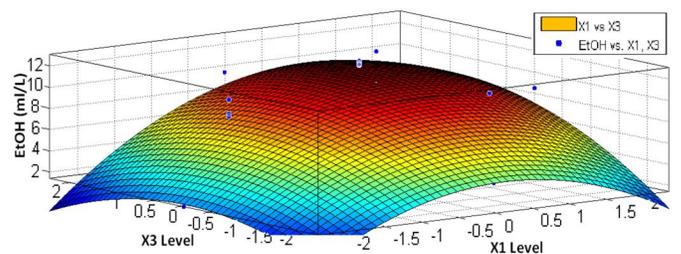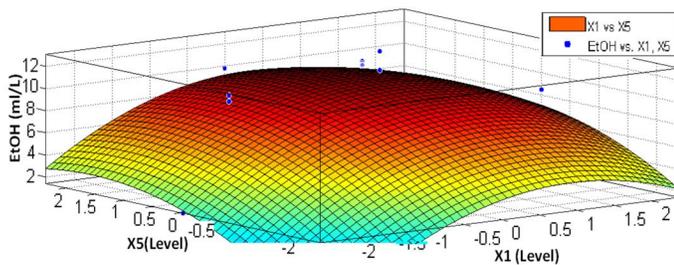

(a) Response Surface
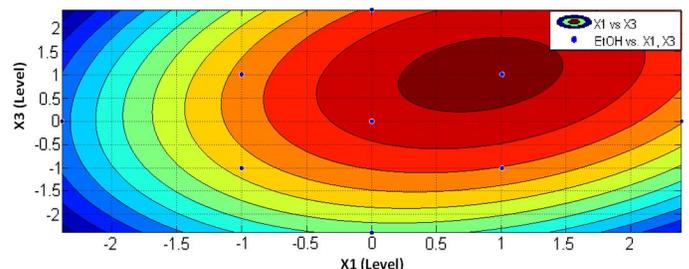
(b) Contour Plot

Fig. 2. EtOH vs X_1, X_3

Fig. 2 above shows the response surface and contour plot of the interaction between substrate concentration
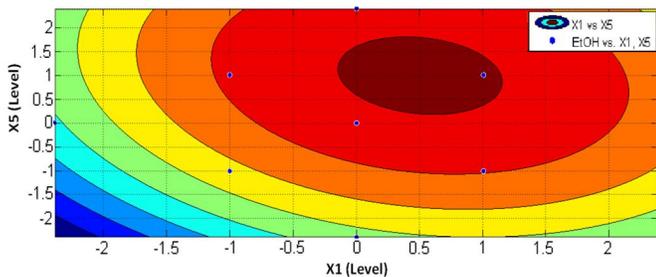
and initial fermentation media pH. The smallest ellipse on contour plot which corresponds to the surface peak shows the maximum predicted ethanol yield. The bottom regions of the surface plot represent minimal ethanol yield. Almost equi-spaced contours shows that this region lies on the exponential phase of the fermentation process while the surface peak lies on the lag stage of invertase activity. Beyond the peak, significant retardation on the response is noted.

High responses for initial substrate concentrations lie between approximately +0.25 (190 g/L) and +1.75 (250 g/L) while those for pH lie between +0.25 (5.75) and +1.5 (7.00). To achieve maximum yield, it is required that both of these factors be set within the above domains.
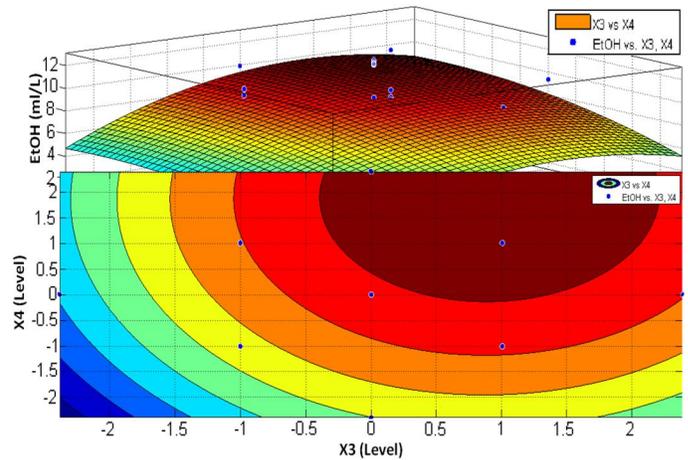

(a) Response Surface

(b) Contour Plot

Fig. 3. EtOH vs X_1, X_5

Fig. 3 above shows the response surface and contour plot of the interaction between substrate concentration and incubation periods. The smallest ellipse on contour plot which corresponds to the surface peak shows the maximum predicted ethanol yield. The bottom regions of the surface plot represent minimal ethanol yield. Nearly equi-spaced contours shows that this region lies on the exponential phase of the fermentation process while the surface peak lies on the lag stage of invertase activity. Beyond the peak, significant retardation on the response is noted.

High responses lie between approximately -0.25 (170 g/L) and +1.25 (230 g/L) initial substrate concentrations and +0.25 (126 hours) and +1.75 (162 hours). To achieve maximum yield, it is required that both of these two factors be set within the above domains.
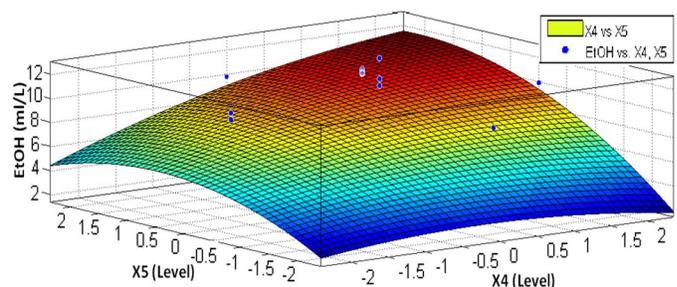

(b) Contour Plot

Fig. 4. EtOH vs X_3, X_4

Fig. 4 above shows the response surface and contour plot of the interaction between initial media pH and yeast concentrations. The smallest ellipse on contour plot which corresponds to the surface peak shows the maximum predicted ethanol yield. The bottom regions of the surface plot represent minimal ethanol yield. Nearly equi-spaced contours shows that this region lies on the exponential phase of the fermentation process while the surface peak lies on the lag stage of invertase activity. Beyond the peak, significant retardation on the response is noted.

High responses lie between approximately -0.5 (5.00) and +2.25 (7.75) initial media pH and from +0 (2 g/L) yeast concentration. To achieve maximum yield, it is required that both of these two factors be set within the above domains.

Fig. 5 below shows the response surface and contour plot of the interaction between initial media pH and incubation periods. The smallest ellipse on contour plot which corresponds to the surface peak shows the maximum predicted ethanol yield. The bottom regions of the surface plot represent minimal ethanol yield. Nearly equi-spaced contours shows that this region lies on the exponential phase of the fermentation process while the surface peak lies on the lag stage of invertase activity. Beyond the peak, significant retardation on the response is noted.
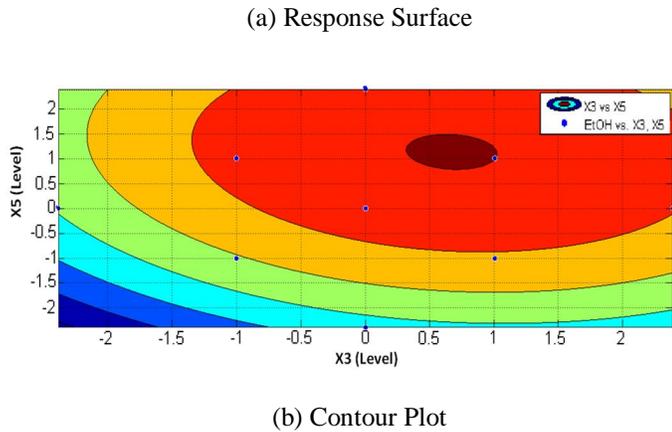
(a) Response Surface

(b) Contour Plot

Fig. 5. EtOH vs X_3, X_5

High responses lie between approximately +0.25 (5.75) and +1.00 (6.50) initial media pH and +0.25 (126 hours) and +1.50 (156 hours). To achieve maximum yield, it is required that both of these two factors be set within the above domains.
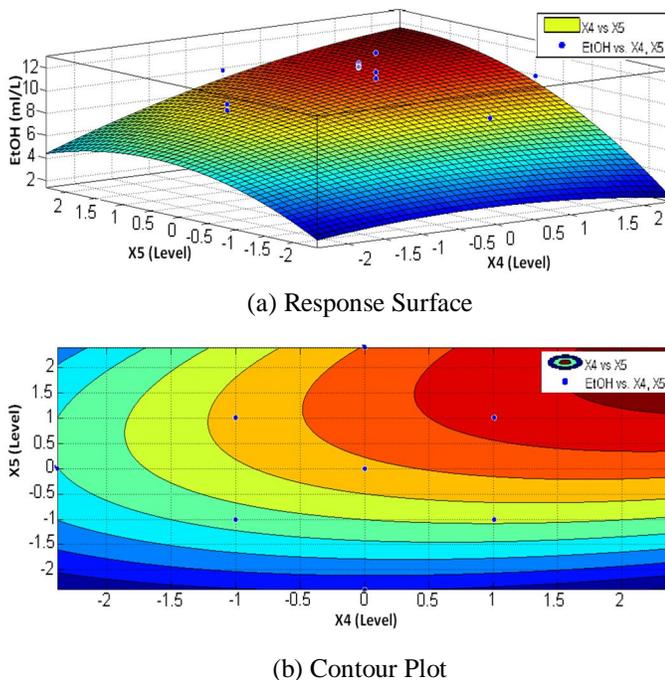

(a) Response Surface

(b) Contour Plot

Fig. 6. EtOH vs X_4, X_5

Fig. 6 above shows the response surface and contour plot of the interaction between yeast concentrations and initial media incubation periods. The smallest ellipse on contour plot which corresponds to the surface peak shows the maximum predicted ethanol yield. The bottom regions of the surface plot represent minimal ethanol yield. Nearly equi-spaced contours show that this region lies on the exponential phase of the fermentation process while the surface peak lies on the lag stage of invertase activity.

Beyond the peak, significant retardation on the response is noted.

High responses lie approximately at substrate concentrations equal or greater than +1.5 (2.75 g/L) and incubation periods equal or greater than +1.00 (144 hours). To achieve maximum yield, it is required that both of these two factors be set within the above domains.

Effect of temperature on bioethanol production was also significant alongside other main effects. Highest achievable enzyme activities were assumed to correspond to the highest bioethanol yield of 13.09 ml/L at 35°C incubation temperature in *Ngombe*. Productivity of invertase highly relies on temperature [12]. Further incubation temperature increase resulted in low bioethanol production which was attributed to reduced invertase productivities. These higher temperatures inactivate yeast culture were thus not conducive for yeast growth [12]. Thermo-tolerant strains of yeast are the best at high incubation temperatures.

Effect of initial fermentation media pH on bioethanol production is was similarly considered closely. Some researchers have realized optimum pH of 5.00. Inhibitory effect of pH above the optimum values realized in this research could be due to reduced formation of ATP across metabolic changes in the yeast [12].

Increase in yeast cell increase bioethanol production. Yeast was used as the main producer of sugar degradation enzymes. Maximum bioethanol yield corresponded to a yeast culture loading containing 2g per litre of the fermentation broth. Yeast cells might have probably become inactive and died at 120 hours incubation. These yeast cells might have tolerated this maximum ethanol concentration of 13.09 ml/L beyond which they died thus contributing to this optimum yield.

Very clear incubation temperature effect at rising substrate concentrations were exhibited in the study. Production of invertase enzymes rose and then declined with severity of either factor. The remaining carbon source (sugars) was used in cell maintenance and ATP generation [13]. There was high substrate concentration in the broth due to fewer yeast cells consuming it. Bioethanol production closely coincided with TRS degradation in various experimental runs.

4. Conclusions

Massive requirement of bioethanol as a renewable energy resource and proper agro-waste management demands efficient and sustainable technologies. This study indicates that viable amounts of bioethanol could be obtained by fermenting banana peels using *Saccharomyces cerevisiae*. RSM was very efficient, cheap and fast in the optimization of these anaerobic fermentation factors. The potential of bioethanol

production using banana peels of this common *Ngombe* cultivar was found to be high. However, the method of still batch fermentation is associated with low conversions due to accumulation of toxic products as well as other by-products in the fermentation broth. This maximum bioethanol yield can be further improved by employing superior bioreactors and fermentation techniques such as the continuous fed fermentation using the mathematical model developed above.

Acknowledgements

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