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Optimization of bioethanol production from nigerian sugarcane juice using factorial design

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Abstract. The quest to reduce the level of overdependence on fossil fuel product and to provide all required information on proven existing alternatives for renewable energy has resulted into rapid growth of research globally to identify efficient alternative renewable energy sources and the process technologies that are sustainable and environmentally friendly. The present study is aimed at production and characterization of bioethanol produced from sugarcane juice using a 2^4 factorial design investigating the effect of four parameters (reaction temperature, time, concentration of bacteria used and amount of substrate). The optimum bioethanol yield of 19.3% was achieved at a reaction temperature of 30°C, time of 72 hours, yeast concentration of 2 g and 300 g concentration of substrate (sugarcane juice). The result of statistical analysis of variance shows that the concentration of yeast had the highest effect of 7.325 and % contribution of 82.72% while the substrate concentration had the lowest effect and % contribution of -0.25 and 0.096% respectively. The bioethanol produced was then characterized for some fuel properties such as flash point, specific gravity, cloud point, pour point, sulphur content, acidity, density and kinematic viscosity. The results of bioethanol characterization conform to American society for testing and materials (ASTM) standard. Hence, sugarcane juice is a good and sustainable feedstock for bioethanol production in Nigeria owing relative abundance, cheap source of supply and available land for large scale production.

Keywords: bioethanol; production; statistical analysis; factorial design; parameters

1. Introduction

Energy is an index for global technological advancement. The present world energy supply derived from non renewable source cannot satisfy the increasing world demand arising from population explosion and the rapid depletion of the source of non renewable energy source (García *et al*. 2011, Quinteroa *et al*. 2008, Kim 2014). The global climate changes resulting from

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Table 1 Variation of parameters of the 2^4 factorial design

Parameters	Level 1	Level 2
Time (Hrs)	72	96
Temperature (°C)	30	40
Feed Ratio	200:1	300:1
Fungi Concentration (g)	1	2

Nigeria. Its juice has sufficient minerals and organic nutrient that makes it suitable for the production of ethanol (Limtong *et al*. 2007, Karuppaiya *et al*. 2012). The complexity of the production process depends mainly on the feedstock being used (Escobar *et al.* 2009, Sanchez and Cardona 2008). The sugars content of the cane do not require modification during fermentation (Ranković *et al*. 2009). However, the optimization of bioethanol produced from Nigerian sugarcane has not been adequately investigated. This study focuses on the optimal production and characterization of bioethanol from sugarcane juice through fermentation using *Saccharomyces Cerevisiae* as the fermenting organism.

2. Materials and method

2.1 Juice extraction and pre-treatment

The sugarcane stem were cut and shredded into pieces and the bagasse was taken to a hydraulic press to extract juice which was separated from the bagasse. A known amount of sugarcane juice was measured and transferred into a beaker. Lime juice was added to it serve as a flocculants which coagulated the impurities present in the juice. The content in the beaker was then allowed to settle for 5 hours, the treated juice was decanted to remove impurities.

2.2 Production of bioethanol from sugarcane juice

Sugarcane juice was used for the production of bioethanol via direct fermentation with the aid of fermenting organism (Saccharomyces Cerevisiae) under anaerobic condition (absence of oxygen). The specification from the 2^4 factorial designs was followed accurately to determine the combination of parameters that can give the possibly highest yield for the production of bioethanol from sugarcane juice. The 2^4 factorial design was employed which implies that four factors were studied at 2 levels as shown in Table 1. The variables were temperature, time, and ratio of load and concentration of fungi used for sixteen consecutive runs. The variables were varied to determine the highest yield of production.

2.3 Sugarcane juice characterization

The properties measured for the juice include moisture content, brix (sugar content), viscosity, refractive index, and its density.

2.3.1 Determination of moisture content

2.4.2 Distillation characteristics

Heat was applied to the distillation flask content gradually and the initial boiling point (IBP) was observed and recorded, with the tip of the condenser away from the wall of graduated cylinder. The graduated cylinder was moved immediately so that the tip of the condenser touches the inner wall. The heating process was regulated such that the time taken from initial boiling point to when 10% of the sample (by volume) was recovered and noted and the temperature at which this occurs was read on the thermometer and recorded accordingly. The heating was continuously regulated so that the uniform average rate of condensation for 10%-99% recovered was obtained. In the interval between the initial boiling point and ends of the distillation, all volumes in the graduated cylinder and all thermometer readings corresponding to them were recorded. The end point, which is the final boiling point (FBP) was observed and recorded. While the condenser tube continues to drain into the graduated cylinder, the volume was measured accurately and recorded. After the flask has been cooled, its content was poured into a 5ml graduated cylinder. The flask was allowed to drain until no appreciable increase in the volume of the liquid in the 5ml graduated cylinder was observed. The values obtained for the percent recovery was added to the percent residue and the total recovery was obtained.

2.4.3 Determination of pour point

The bioethanol was poured into test jar to the appropriate level. The cork into which the thermometer was inserted tightly into closed the test jar, the position of the cork was adjusted and the thermometer fits the cork tightly. The thermometer and the cork were set coaxial and the thermometer bulb was immersed such that one end of the capillary was 3mm below the surface of the juice. The bioethanol was heated without stirring to 58°C and maintained at this temperature. The fuel was cooled to 35°C (95°F) in water bath. A jar ring was placed around the testing jar 25 mm from the bottom. The test was inserted into the ice jacket. The jacket was supported by the test jar in a vertical position in the cooling bath. After preliminary heating, the sample was cooled at a specific rate and examined an interval of 2°C for flow characteristic. The lowest temperature at which movement of the ethanol was observed was recorded as the pour point.

2.4.4 Determination of cloud point

Sample of bioethanol was placed in a test jar to a mark and then placed inside a cooling bath. The temperature at the bottom of the test jar that is the temperature at which the bioethanol starts to form cloud was taken as the cloud point.

2.4.5 Determination of ethanol concentration

This was determined using a refractometer with the aid of the refractive index method. The refractive index value is cross reference to the standard table of ethanol concentration and the value was then confirmed.

2.4.6 Determination of ash content

The sample was put on a metal plate and placed over an ignited burner until the entire organic matter was charred. It was transferred to a muffle furnace and maintained at 550°C for a few hours until grey ash was obtained, after which it was cooled in a desiccator. The ash residue was weighed and values recorded (Food Safety and Standards Authority of India, 2012). The % ash content of the bioethanol was calculated as the ratio of mass of the ash to the mass of the sample.

Table 3 Percentage yield of bioethanol produced

Runs	Temperature (O^C)	Time (Hrs)	Conc of Fungi (g)	Feedstock (g)	Yield (%)
1	30	72	1	200	8.2
2	30	72	1	300	7.7
3	30	72	2	200	13.6
4	30	72	2	300	19.3
5	30	96	1	200	8.9
6	30	96	1	300	6.2
7	30	96	2	200	13.7
8	30	96	2	300	15.4
9	40	72	1	200	7.6
10	40	72	1	300	6.7
11	40	72	2	200	13.0
12	40	72	2	300	14.6
13	40	96	1	200	9.0
14	40	96	1	300	5.0
15	40	96	2	200	15.6
16	40	96	2	300	12.7

Limtong 2007), fungi aid fermentation effectively within the range of 4-6. The difference in pH values with other reported works (Garcia *et al*. 2011) can be associated to the type of feedstock, pre-treatment method employed and the geographical location where feedstock were obtained from (Iye and Bilsborrown 2013, Suleiman *et al*. 2014).

3.2 Yield of bioethanol

Table 3 shows the optimal percentage yield of bioethanol produced with respect to each variable investigated (temperature, time, fungi concentration and feed stock). The result shows that an optimal yield of 19.3 was obtained at a substrate concentration of 300 gram, fungi concentration of 2 gram, fermentation period of 72 hours and a temperature of 30°C.

3.3 Fuel properties of bioethanol

The result of the properties of produced bioethanol and previously reported works is presented in Table 4.

3.3.1 Flash point

This is a key property in determining the flammability of a fuel. The flash point is the lowest temperature at which an applied ignition source causes the vapours of fuel to ignite. It is therefore the tendency of a sample to form flammable mixture (Graeme and Walker 2010). The flashpoint of ethanol produced was 17°C which is shows close proximity to 16.60°C reported in literature (García *et al*. 2011), but lower than ASTM minimum value of 18.60 and ≤21°C reported in literatures (Buraimoh 2014). The higher the flash point the safer the fuel in terms of handling,

3.3.5 Pour point

The pour point is an important characteristic of the bioethanol that says the lowest operational temperature of the bioethanol. The pour point was determined according to ASTM D97. The value obtained was 4.0°C. The result obtained was lower than 5.20°C stipulated by the ASTM standard. The value obtained is a clear indication that the bioethanol can fully serve its efficiently and satisfactorily be used even in polar regions where the atmospheric or room temperature is not less than 4.0°C.

3.3.6 Sulphur content

Sulphur in the atmosphere has been associated with negative impacts on human health and on the environment. Mutagenic potentials have been ascribed to sulphur dioxide and particulate matters emitted by automobiles operating on high sulphur-containing fuels. Because of these reasons, there is currently a strict tightening of international limits. Bioethanol fuels have traditionally been acknowledged as sulphur-free and this has been accounted as one of its greatest advantage over fossil fuel and the results obtained in this work attested to that fact. The sulphur content reported for the ethanol produced was 1.58 which compared adequately but a little bit higher than that obtained for bioethanol synthesized in Chile (García *et al.* 2011).

3.3.7 Acidity

The acidity value of the fuel is dependent on a number of factors which include the type of feedstock used for the fuel production, production process and its respective degree of purification (García *et al.* 2011). The acidity value of the fuel was 0.38. Corrosion of chromium and zinc parts within the engine and injection system has been linked with high acidity of the fuel (Limtong *et al*. 2007). The low value obtained clearly depicts that the fuel will not pose corrosion danger during storage or damage to engine parts.

3.4 Effect of process parameters on ethanol production

The effect of process condition on bioethanol production yield from sugarcane juice was

Table 4 Properties of bioethanol produced from sugarcane juice

Property	Units	1	Reported Work		2 (Gasoline)	ASTM Standard	Present Work
Density	gcm^{-3}	0.789	0.792	0.789	0.74	0.790	0.789
Specific Gravity	-	0.789	0.879	-	59.53	0.87	0.789
Kinematic Viscosity	mm^2s^{-1}	-	-	-	0.487	5.0 max	3.80
Boiling Point	°C	78.50	78.4	78.4		78.50	78.00
Flash Point	°C	-	12	≤ 21		18.60	17.00
Cloud Point	°C	-	-	-	-22	23.00	10.00
Water Content	-	≤ 0.3	-	-		-	2.70
Pour Point	°C	-	-	-		5.20	4.0
Sulphur content	(wt %)	-	-	-		-	1.58
Acid Value			-	-		-	0.36

1,2. Kheirilla *et al*. (2012); Reported works: Buraimoh (2014)

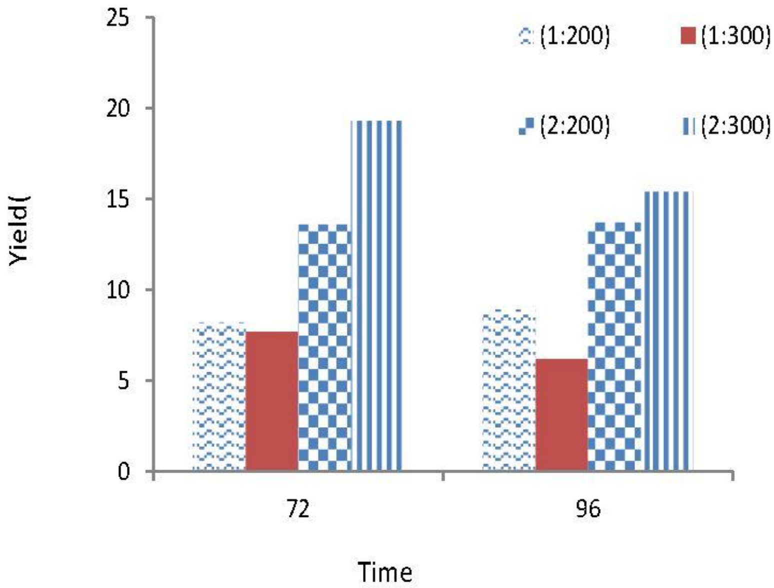

Fig. 2 Effect of reaction time on bioethanol yield at various feed loading

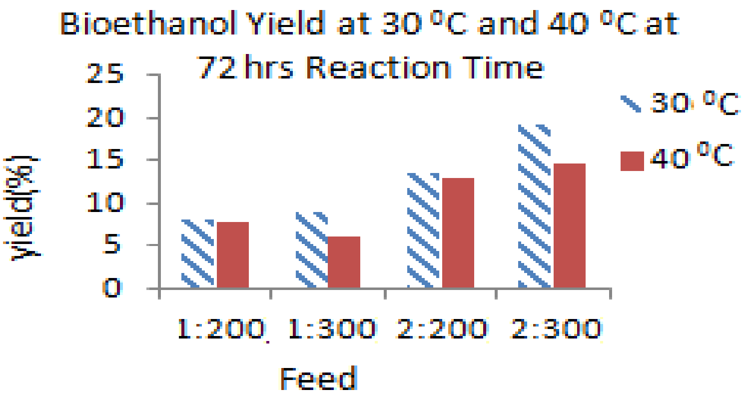

Fig. 3 Effect of feed ratio on bioethanol yield at various temperatures

In this study, 72 and 96 hrs was chosen as low and high level respectively. From the result presented in Table 3 the bioethanol yield of 19.3% obtained after 72 hrs was greater than 15.4% obtained after 96 hrs. This result shows quantitative agreement with the some reported works (Sobrinho *et al*. 2011, Pimpakan *et al*. 2012, Achigan-Dako *et al*. 2010, Li *et al.* 2008). A close experimental observation of the fermentation process after a fermentation period of 96 hours revealed the appearance of a whitish clayish substance around the conical flask which was suspected to be a death in fungi as a result of degradation in nutrient concentration (Larissa *et al.* 2013).

3.4.3 Effect of feed ratio (Fungi conc.: amount of sugarcane juice)

The result presented in Fig. 3 shows that the higher the feed ratio, the higher the yield of

Table 6 Summary of ANOVA on the 2^4 Fermentation Experiment

Source	Sum of Square	Df	Mean Square	F Value	P Value (Prob>F)
MODEL	259.44	14	18.53	1853.14	0.0182
A-Temperature	4.84	1	4.84	484.00	0.0289
B-Time	1.10	1	1.10	110.25	0.0604
C-Fungi Conc.	214.62	1	214.62	21462.25	0.0043
D-Feedstock	0.25	1	0.25	25.00	0.1257
AB	1.56	1	1.56	156.25	0.0508
AC	0.72	1	0.72	72.25	0.0746
AD	6.76	1	6.76	676.00	0.0245
BC	0.25	1	0.25	25.00	0.1257
BD	11.90	1	11.90	1190.25	0.0184
CD	12.60	1	12.60	1260.25	0.0178
ABC	1.00	1	1.00	100	0.0635
ABD	0.12	1	0.12	12.25	0.1772
ACD	3.06	1	3.06	306.25	0.0363
BCD	0.64	1	0.64	64.00	0.0792
Residual	1.000E002	1	1.00E-002		
Correlative Total	259.45	1			

0.096%. The results presented deduced that the interaction effect between the factors were very significant. Other combination parameters like CD (concentration of fungi and ratio of juice used) had effect of -1.775 with percentage contribution of 4.86%. ABCD (temperature, time, concentration of fungi, and ratio of feed) had effects of 0.05 but the lowest percentage contribution of 0.0039%. AC, AD, BC. ABD, ACD, BCD, all have negative effects of -0.43, -1.3, -0.25, -0.173, -0.173, -0.88, -0.4 with percentage contribution of 0.28, 2.61, 0.096, 4.59, 0.047, 1.180 and 0.247% respectively. The Fishers-value of the model is 1853.14 as shown in Table 6 and it implies that the model is significant and there is only a 1.82% chance that a model "F-value" is this large could be due to noise (different disturbances). Values of 'prob>F' less than 0.0500 indicate model terms significance. In this case, A, C, AD, BD, CD, ACD are significant model terms because each is greater than 0.1000.

3.6 First degree regression model

From ANOVA, it can be concluded that A, B, D, C were significant factors. The mathematical equation models for predicting average bioethanol yield are final Eqs. (4) and (5) given interms of coded and actual factors. Fig. 4 shows the graph of predicted versus the actual yield of bioethanol obtained from either of the two model equations The graph shows a better correlation between the actual and predicted yield obtained and reflects the value of R^2 obtained as 0.9901.

$$Y=11.07-0.55A-0.26B+3.66C-0.12D+0.31A*B-0.212A*C-0.65A*D-0.13B*C-0.86B*D+0.89C*D+0.25A*B*C-0.087A*B*D-0.44A*C*D-0.20B*C*D \quad (4)$$

$$Y=26.62500-0.64500A+0.11875B-16.95000C-0.19750D+0.000A*B+0.09000A*C+5.10000E-003A*D-0.14583B*C+5.83333E-004B*D+0.21400CD+8.33333E-003A*B*C-2.91667E-005A*B*D-3.50000E003A*C*D-0.660667E-004B*C*D \quad (5)$$

However, the result preseted in Figs. 5 to 8 shows the effect of each of the factors (Temperature, Fungi concentration, feedstock, and time) on the yield of bioethanol produced. The one factor analysis in Figs. 5 and 6 on the yield shows that the yield of bioethanol decreases as the temperature and time increases from the optimal value of 30°C and 72 hours respectively. The high level temperature and time selected in the optimization process from the literature have the least percentage yield of bioethanol.

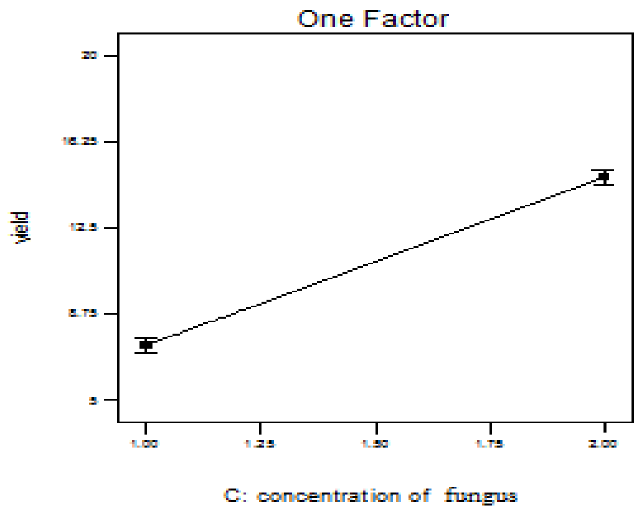

Fig. 7 Graph of yield (%) against concentration of fungi (g)

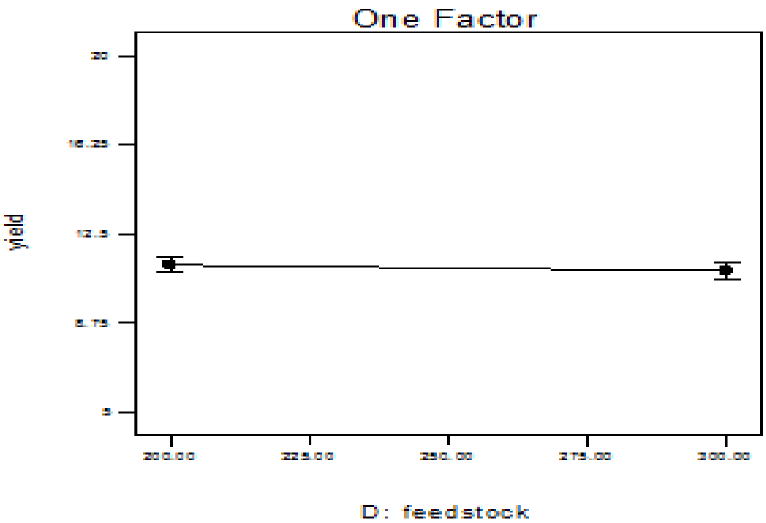

Fig. 8 Graph of yield against amount of feedstock used

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