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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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atmospheric pollution is the key consequences associated with the use of petroleum derived fuels (Quinteroa *et al*. 2008, Kim 2014).

Renewable energy is widely acknowledged as the fastest growing energy source due to its environmentally friendly nature and renewability (Maity *et al*. 2014). According to the international energy agency's (IEA), the world demand for renewable energy is expected to grow continuously between 2007 and 2030 at a compound average rate of 7.3% annually (Sadorsky 2011). Bioethanol production level in the world has been identified to account for about 10% of the world energy produced (Campbell and Doswald 2009, Balat and Balat 2009). Bioethanol has been identified as one of the most common liquid biofuel that can be produced from sugar (sugar cane, sugar beets and sorghum), starchy (maize, wheat, barleys etc.) or cellulosic (crop residues, hard wlood, softwood) raw materials (Ko *et al*. 2012, Zegada-Lizarazu and Monti 2012). Among these feedstock wheat, barley, corn, potato, cassava, sugarcane, sugar beet are examples of first generation biofuel feedstock; whereas, cassava waste (roots, peels, stem and leaves), Miscanthus, straw, wood, and grass constitute the sources of second generation biofuels raw materials (Musa 2012, Bala 2014).

Today, many countries have outstanding record of bioethanol production. The major producers are Brazil, USA and China with production capacity of 18.3, 17.5 and 1 billion litres respectively as at 2006 (Julián *et al.* 2011). The production trends of these leading countries shows that there is an increasing interest in identifying any possible renewable source. Sugar and starchy raw materials are presently the major feedstock of bioethanol production (Theuretzbachera *et al.* 2013, García *et al.* 2011). In United States corn grain is the primary feedstock used for fuel ethanol production. This country has a total production capacity of about 13.3 billion gallons as at 2012. The production of bioethanol in U.S favours the Midwest corn-belt states in which the ethanol production plants are mostly located. There were only 50 ethanol plants in 2000 according to RFA (Renewable Fuel Association) and these numbers rapidly increases to 198 in 2011. However, it was observed that the number of plants under construction in 2005 was 76 plants and this number dropped sharply to seven in 2011 which is an indication that the ethanol demand in the US appears to have reaches its saturation state and government mandates on corn-starch ethanol has only 3 years, approximately 12% to reach maximum (Kim 2014). In Brazil, bioethanol is produced from sugarcane juice (Larissa *et al.* 2013). Apart from Brazil other world leading countries of bioethanol that focus on the use of sugarcane include India and Australia (Koçar and Civaş 2013).

The Nigeria's production capacity of 1st generation bioethanol currently stood at 134 000 m^3 per annum coming from five major commercial scale ethanol distilleries located in Lagos, Sango-Ota and Bacita. In order to meet the nation's local domestic demand of 5.14 Mm^3 per annum, over $3.86 billion has been invested in the feedstock plantation and plant construction of 19 ethanol bio-refineries with an expected annual capacity of over 2.66 Mm^3 of fuel grade ethanol annually (Iye and Bilsborrown 2013). The target feedstocks are mainly sugar cane, cassava and sweet sorghum. However, to meet the 10% ethanol replacement (E10) in petroleum motor spirit will require about 1 million hectares of land which is 3% of the 34 million hectares under cultivation (Iye and Bilsborrown 2013). These land under cultivation represent only about 8% of the Nigeria's arable land underutilized with potentials of providing in excess of Nigeria's and West Africa food demand. The use of particular crop depends on its domestic availability and level of production in addition to sustainability, favourable soil and climatic conditions of the region peculiar to the energy crop (Escobar *et al.* 2009). Today, sugar cane is the best known crop for the production of biofuel with high biomass content of about 12-17% total sugars constituted by 90% sucrose and 10% glucose/fructose (Limtong *et al*. 2007). Sugarcane is widely grown in the northern region of

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

Moisture content was determined by the oven drying method by sequentially drying at 70-80°C for 2 hours followed by final drying at 105°C for 4 hours till constant weight was reached.

2.3.2 Brix (Sugar content)

Sugar cane is the best known crop for the production of biofuel with high biomass content of about 12-17% total sugars made up of 90% sucrose and 10% glucose/fructose. The method of determining the quantity of these sugars is contained in literature as reported (Limtong *et al*. 2007)

2.3.3 Determination of viscosity

A cleaned, dried viscometer with a flow time above 200 seconds for the fluid to be tested was selected. The sample was filtered through a sintered glass to eliminate dust and other solid materials in the liquid sample. The viscometer was charged with the sample by inverting the tube's thinner arm into the liquid sample and sunction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a constant temperature bath set at 40°C and allowed approximately 10 minutes for the thinner arm to draw the sample slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flow freely from the upper timing mark to the lower timing mark was recorded (Limtong *et al*. 2007).

2.3.4 Determination of refractive index

In accordance to (Limtong *et al*. 2007), refractive index was determined with the aid of refractometer. Few drops of the sugarcane juice sample was placed on the refractometer and allowed to gently spread close and it was tightened for 20 seconds as to allow the juice and the prism attain a steady temperature. After adjusting to where it coincided with the diagonal crossing when looking through the lens, the refractive index was read from the demarcation.

2.3.4 Determination of density

The density was measured in comparison to water by the standard gravimetric method.

2.4 Characterization of bioethanol

The bioethanol produced from sugarcane juice was characterized for some of its fuel properties such as flash point, distillation characteristics, pour point, cloud point, ethanol concentration, ash content, water content and viscosity based on the reported method (Ademiluyi 2013).

2.4.1 Determination of flash point

Pensky Martens (ASTMD93) method was used to determine the flash point of the bioethanol. The sample to be tested was placed in a brass cup in such a quantity as to just touch the prescribed mark on the inside of the cup. The cover was then fitted into position on the cup. The Bunsen burner was used to provide heat to the lower side of the apparatus. The heating was adjusted to provide a temp rise of about 7°F per minute, and the sample was continuously stirred. As the sample approach the temperature of the flash, the injector burner was lighted on and then injected into the sample at about 12 seconds interval until a distinct flash was observed within the container and the injector burner put off. At this point the close flash point was noted with the aid of a thermometer. The flash point was then recorded.

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 2 Chemical properties of sugarcane juice (Data represent average)

Parameters	Unit	Values
Moisture content	(%)	85.05
Density	(kg/m^3)	4.459
Brix(Sugar content)	(%)	18
pH		5.55

2.4.7 Determination of water content

A known weight of the sample was heated at a constant temperature of 100°C in an oven for 50 minutes and weight was taken at every 10 minutes. The process was repeated until a constant weight was obtained. After every 10 minutes the sample was removed and placed in desiccators for 20 minutes to cool. The sample was then removed and re- weighed. The percentage of water content was then calculated.

2.4.8 Determination of sulphur content

The sulphur content of bioethanol produced was determined using ASTM.D2622 method. The sample was poured into a disposable container to fill up to three quarter its capacity in order to ensure the passage of X-ray through it sufficiently thereby enabling sufficient sulphur content. The sample was then covered with X-ray transparent plastic film window. This followed by switching on the power source which lights using the X-ray lamp within seconds. Three different readings were provided at an interval of 30seconds. The readings recorded and average sulphur content was determined in percentage sulphur weight. The viscosity of the bioethanol was determined using similar procedure for the treated sugarcane juice.

3. Results and discussion

3.1 Sugarcane juice characterization

Table 2 presents the result of characterization of major properties of raw treated sugarcane juice measured repeatedly and average of each parameter investigated is presented.

Moisture content is one of the basic properties to be tested for in any material to be utilized as a feedstock for bioethanol production. It has been reported that for sugarcane juice to be suitable for production of bioethanol, the moisture content must be within the range of 80-85% value higher than the set standard limit will alter the efficiency of the fermentation process (Andrietta 2009, Limtong 2007, Abdullahi 2013). Result presented in Table 2 indicates that the moisture content of sugarcane juice to be used as a feedstock for bioethanol production is 85.05% which is higher than the set limit. Hence the need to dehydrate the sugarcane juice before subsequent fermentation of the sugarcane juice to bioethanol.

Brix is the amount of sugar content present in a sugarcane juice sample. The sugar content present in this sample was determined to be 18%. This value falls within the range of 14-22% reported for bioethanol production (Limtong 2007). The pH value of the juice was also determined to be 5.55 as presented in Table 2. The appreciable pH value obtained provide enabling environment for the use of fungi in the fermentation process. According to (Andrietta 2009,

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,

storage, and transportation (Gerpen *et al*. 2004). Balat and Balat (2009) added that the flash point of bioethanol permit higher compression ratio and reduced combustion period, as obvious reason for better efficiency in internal compression engine over petroleum derived gasoline. The result obtained shows that the bioethanol produced do not pose hazard at low temperature during storage.

3.3.2 Density and specific gravity

Density is an important parameter for ethanol fuel injection systems. The value of density must be maintained within the tolerable limits to allow optimal air to fuel ratios for complete combustion. High density bioethanol can lead to incomplete combustion and particulate matter emission (Limtong *et al*. 2007, Balat and Balat 2009). The density of bioethanol produced was determined to be 0.789 gcm^{-3}. This value is in accordance with some reported literature but shows slight deviation from 0.792 reported in the same work (Buraimoh 2014). The value is also higher than 0.74 reported (Kheiralla *et al*. 2012). It is important to state that the slight disparity in density observed can be strongly attributed to differences in feedstock used, fermentation process employed and presence of impurities. Specific gravity is the ratio of the density of an observed substance to the density of a reference substance (mostly water) at the same conditions (Ajav and Akingbehin 2002). It is a very important property of bioethanol which has relevance in blending with gasoline. This property also impact positively on the efficient performance of engine, since fuel injection a system operates on volume metering basis (Demirbas 2009). The specific gravity of ethanol produced was 0.789 which shows appreciable consistency with the literature (Kheiralla *et al*. 2012) but slightly lower than the ASTM standard value. The result shows that sugar juice based bioethanol is lighter than water and will be miscible when in contact with it.

3.3.3 Cloud point

Cloud point is the temperature at which a cloud of crystals first appears in a liquid cooled under a prescribed test condition. It provides information on the low temperature usability of a fuel under extremely cold conditions. The cloud point of ethanol produced was 10°C which is lower than 23 reported for ASTM standard (Graeme and Walker 2010). The low cloud point from this study is an indication that the fuel will perform satisfactory even in cold climatic conditions since the tendency for gel formation was low. However higher cloud point can affect the engine performance and emission adversely under cold climate conditions. The use of bioethanol fuel in an engine at temperatures below the cloud point of the fuel can apparently leads to fuel filter clogging due to the appearance of wax.

3.3.4 Kinematic viscosity

The efficient operability of car engines depends on the kinematic viscosity of the fuel used (Limtong *et al*. 2007, Balat and Balat 2009). The viscosity of a fuel must be given significant consideration for fuel injection combustion chambers system. This property is a measure of the resistance of a substance (mostly liquids) to flow (Kheiralla *et al*. 2012). The viscosity of bioethanol synthesized was 3.80 mm^2s^{-1}. This value is within the ASTM maximum standard value of 5 mm^2s^{-1} stipulated. Appreciable viscosity within set limit must always be maintained to ensure efficient engine functionality. Fuels tends to flow with much ease when its viscosity is excessively low such situation usually have adverse effect as the lubricating film between moving and stationary parts in the carburettor or pump are not maintained. On the other hand very high fuel viscosity hinders the atomization the fuel into small droplets to facilitate good vaporization and combustion (Kheiralla *et al*. 2012).

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)

investigated using 2^4 factorial designs. Four parameters were investigated at two levels. The parameters were temperature, concentration of fungi, time and ratio of sugar cane juice to fungi. Based on the results presented in Table 3, it can be seen that the optimum yield of 19.3% was obtained at the optimum condition of temperature of 30°C, time of 72 hours, concentration of fungi of 2 g and feedstock of 300 g. Result obtained in this study is lower than the optimum yield of 22.73% at 30-34°C and 72 hours fermentation time reported in some works (Buraimoh 2014, Gerpen *et al*. 2004). Analysis of the influence and effects of each parameters investigated on the yield of bioethanol are presented in Table 5.

3.4.1 Effect of temperature

Temperature is a very important factor in any reaction mechanism. This parameter helps in facilitating the rate of certain reactions. Though, fermentation yield of ethanol depends on the ability of the yeast to grow under various temperatures. Two temperatures were considered and results presented in Table 3. In this study, the two levels of temperature are 30°C for the low and 40°C for the high and both below the boiling point of ethanol (78°C). It is very clear from the study that at 40°C, yeast growth might be low and hence ethanol yield was also low. In Table 3, it can be observed that the yield of bioethanol produced at 30°C was 19.3% which is higher than 14.6% obtained at 40°C when the same feed ratio was used. The lower yield observed at this upper temperature limit (40°C) may be attributed to the poor growth of yeast at 40°C and likely thermal decomposition of ethanol (Escobar *et al*. 2009, Pimpakan *et al*. 2012, Achigan-Dako *et al*. 2010). Optimum temperature of 30°C obtained in this study was in agreement with result of literature whose optimum yield was between 30-34°C (Sobrinho *et al*. 2011). The findings as presented also indicate that irrespective of the feed ratio, the yield of bioethanol from sugarcane juice at 30°C was higher than that obtained at 40°C as shown in Fig. 1.

3.4.2 Effect of time

Most available literature on the production of bioethanol from different feedstock reported that complete fermentation takes place at 72 hours (Magdy *et al*. 2011, Chatanta *et al*. 2008). Fig. 2 show the bioethanol yield with time variation.

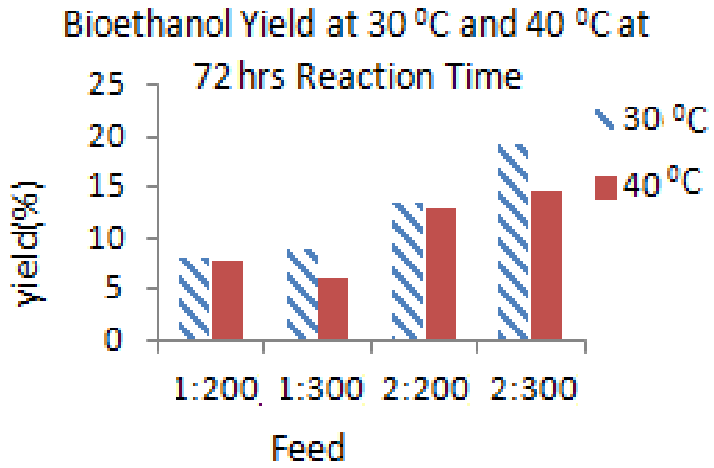

Fig. 1 Effect of temperature on bioethanol yield at various feed ratios

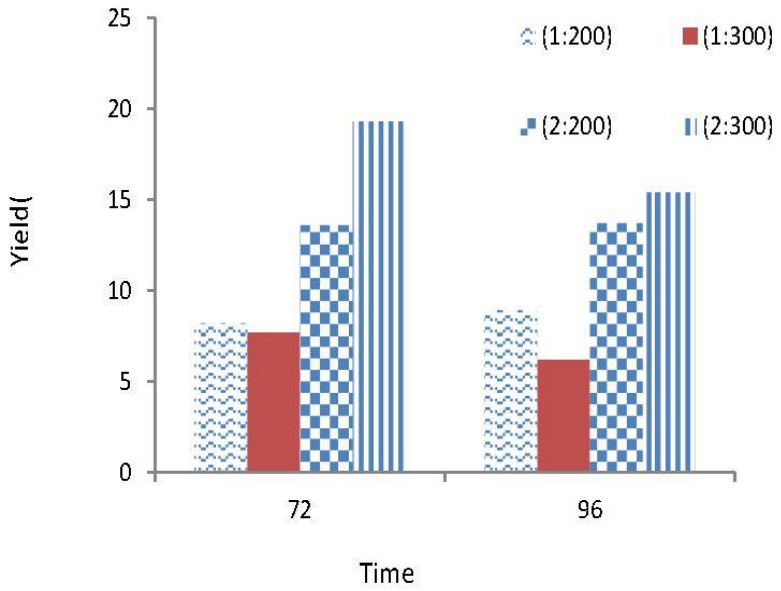

Fig. 2 Effect of reaction time on bioethanol yield at various feed loading

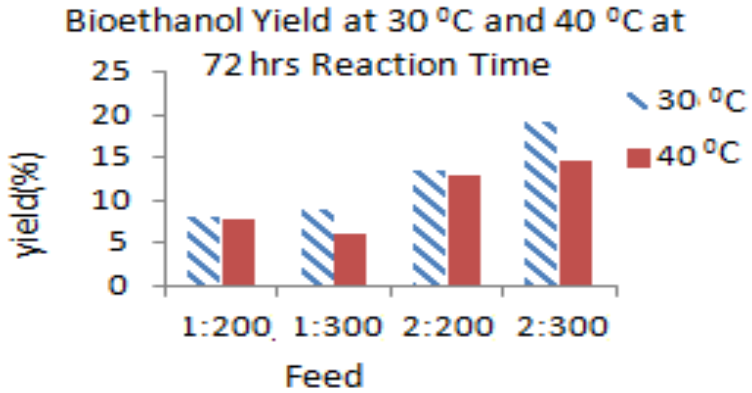

Fig. 3 Effect of feed ratio on bioethanol yield at various temperatures

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 5 Factors effects and contribution

Term Intercept	Effect	Sum Sqr.	% Contr.
A- Temp.	-1.1	4.84	1.87
B- Time	-0.53	1.10	0.42
C- Fungi	7.33	214.62	82.72
D- Feedstock	-0.25	0.25	0.096
AB	0.63	1.56	0.60
AC	-0.43	0.72	0.28
AD	-1.3	6.76	2.60
BC	-0.25	0.25	0.096
BD	-1.73	11.90	4.59
CD	1.78	12.60	4.86
ABC	0.5	1.00	0.39
ABD	-0.18	0.12	0.047
ACD	-0.88	3.06	1.18
BCD	-0.4	0.64	0.25
ABCD	0.05	0.01	0.0039

bioethanol produced. For instance at production time of 72 hours and feed ratio of 2:200, the yield of bioethanol was 13.6%, when the feed ratio was raised to 2:300, the yield of bioethanol was also raised to 19.3%. The results in this study was in agreement with the report of literature which stated that the more the amount of feedstock used the higher the quantity of bioethanol produced (Escobar *et al.* 2009). The optimum yield of 19.3% of bioethanol was obtained at substrate mass of 300 g.

3.4.4 Effect of fungi concentration

The effect of fungi used (saccharomyces cerevaise) is one of the most important factor. The concentrations of fungi used in this study were 1 and 2 g for low and high level respectively. It was observed that the more the concentration of fungi the more the ethanol formation. The highest yield of 19.3% bioethanol was obtained at 2 g of fungi was in accordance with the reported literature (Limtong *et al*. 2007, Taherzadeh and Karimi 2007, Demirbas 2005). The result shown in Table 3 revealed that for all experimental run the ethanol yield increases as the fungi concentration increases from 1 to 2 g.

3.5 Statistical analysis of 2^4 experimental results

Statistical analysis was carried out on the experimental results obtained using analysis of variance (ANOVA). The result of the test of statistical significance in Table 6 shows that the 4 parameters; reaction temperature, time, concentration of fungi used and ratio of feedstock used have their different effect on the bioethanol yield percentage with contribution of 1.87%, 0.43%, 82.72% and 0.096% respectively. It can be observed in Table 5 that the concentration of fungi has the highest effect of 7.325 on bioethanol yield with % contribution of 82.72% while the effect of feedstock ratio of juice used has the lowest effect of -0.25 with the lowest % contribution 0f

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.

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

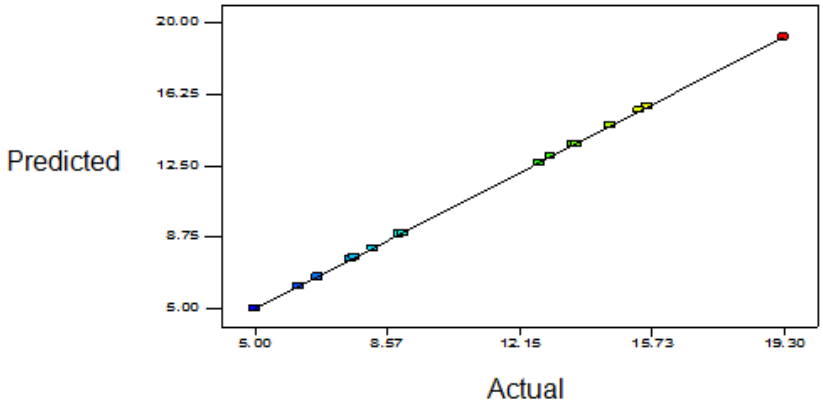

Fig. 4 Graph of predicted versus actual yield

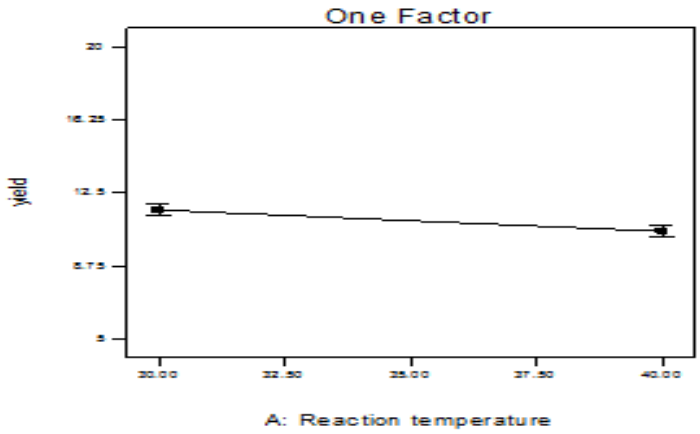

Fig. 5 Graph of yield against reaction temperature

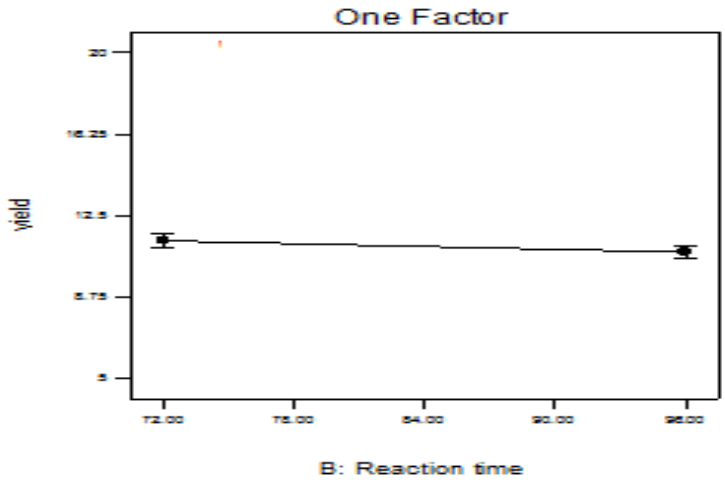

Fig. 6 Graph of yeld (%) against reaction time

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

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.

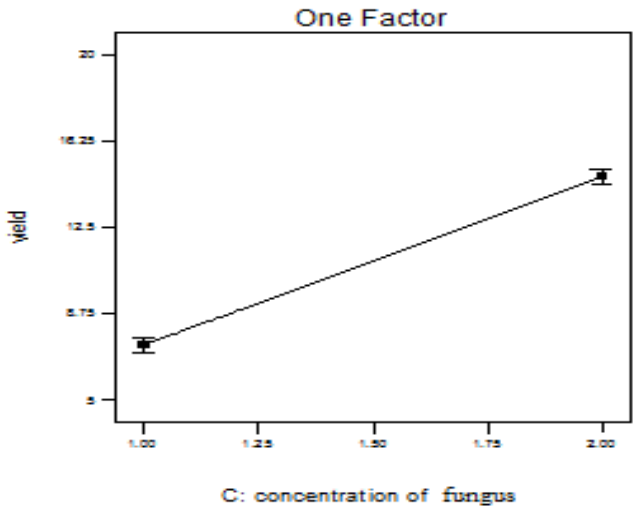

Fig. 7 Graph of yield (%) against concentration of fungi (g)

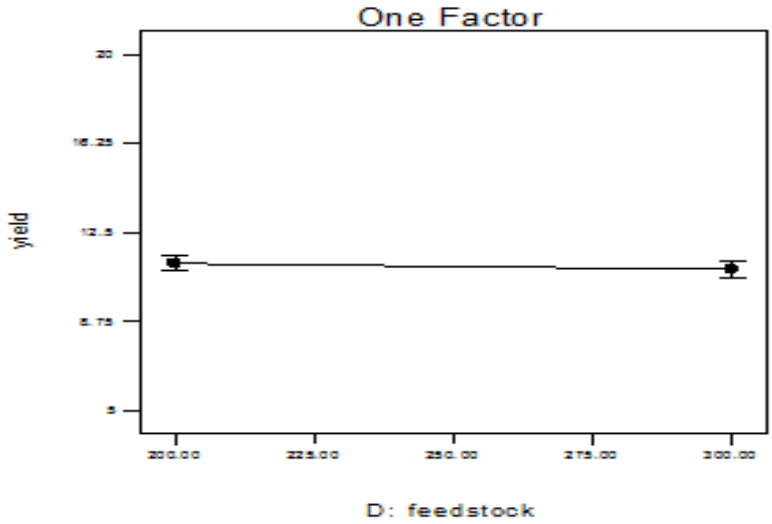

Fig. 8 Graph of yield against amount of feedstock used

Fig. 7 shows a graph of yield (%) against concentrationn of fungi with the concentration of 2 g having the highest yield compared to the concentration of 1 g. The result further indicates that the impact of fungi concentration increase on the overall yield was positive and the greatest when compared to the time and temperature increase whose impact is negative. The factor with little or no impact is the increase in the quantity of feedstock shown in Fig. 8. The increase in feedstock appears to cause an insignificant decrease of the yield which suggest that much supply of feed stock not in the appropriate ratio will only results into a waste.

4. Conclusions

Bioethanol was successfully produced from sugarcane juice using a 2^4 factorial experimental design to investigate the influence of temperature, time, fungi concentration and ratio of feedstock used on the yield of bioethanol production in this study.

• Results revealed that optimum yield of 19.3% of bioethanol was obtained at operating parameters of 30°C fermentation temperature, fermentation time of 72 hours, 2 g of yeast and 300 g of sugarcane juice.

• The juice properties (level of sugar content, low sulphur presence and suitable pH value) show the potentials of Nigerian sugarcane juice in producing desired quality bioethanol.

• The fuel properties conform to the set limit by ASTM and the concentration of fungi had the highest effect and percentage contribution of 7.33 and 82.72 % respectively.

• The production of bioethanol from Nigerian sugarcane juice is sustainable and can serve as a reliable alternative energy source to gasoline.

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References

Ajav, E.A. and Akingbehin, O.A (2002), "A study of some fuel properties of local ethanol blended with diesel fuel: agricultural engineering international", *CIGR J. Scientif. Res. Develop. Manusc.*, **4**, 1-9.

Achigan-Dako, E.G., Pasquini, M.W., Assogba-Komlan, F., N'danikou, S., Yedomonhan, H., Dansi, A., Ambrose-oji, B. (2010), "Traditional vegetables in Benin: diversity, distribution, ecology, agronomy and utilization", Institute National des Recherches Agricoles, du Benin, 252.

Abdullahi, M. (2013), "Optimization of bioethanol production from sugarcane bagasse", M.Eng. Thesis, Federal University of Technology, Minna, Niger State.

Ademiluyi, F.T. (2013), "Yield and properties of ethanol biofuel from different whole cassava flours", *Biotechnol.*, **2**, 1-5.

Andrietta, S.R. (2009), "Optimal Industrial fermentation", *BIOEN Workshop on Process for ethanol production*, FAPESP, Available online http://www.fapesp.br/eventos/2009/09/10_bioen/Silvio_Roberto.pdf, retrieved on Sept. 15, 2009. (in Portuguese)

Bala, E.J. (2014), "Energy and power development in Nigeria: prospects, challenges, and way forward", 21st, 22nd and 23rd Federal University of Technology Convocation Lecture, Minna, March.

Balat, M. and Balat, H. (2009), "Recend trends in global production and utilization of bioethanol fuel", *Appl. Energy*, **86**, 2273-2282.
Buraimoh, S.S. (2014), "Production of Bioethanol from Pinneaple waste using *Zymomonas Mobilis*", B.Eng Thesis, Federal University Of Technology, Minna.
Campbell, A. and Doswald, N. (2009), "The impacts of biofuel production on biodiversity: a review of the current literature", UNEP-WCMC, Cambridge, UK.
Demirbas, B. (2000), "Biofuels for internal combustion engines", *Energy Educ. Sci. Technol*., **22**, 117-32.
Demirbas, A. (2005), "Bioethanol from cellulosic materials: a renewable motor fuel from biomass", *Energy Sour.*, **27**, 32-42.
Demirbas, A. (2009), Characterization of biodiesel fuels", *Energy Sour.*, **31**, 889-896.
Escobar, J.C., Lora, E.S., Venturini, O.J., Yanez, E.E., Castillo, E.F. and Almazan, O. (2009), "Biofuels, technology and food security", *Renew. Sustain. Energy Rev.*, **13**, 1275-128.
García, A.E., Carmona, R.J., Lienqueo, M.E. and Salazarc, O. (2011), "The current status of liquid biofuels in Chile", *Energy*, **36**, 2077-2088.
Gerpen, J.V., Shank, B., Pruszko, R., Clement, D. and Knothe, G. (2004), "Biodiesel production Technology", Subcontractor Report, July.
Iye, E. and Bilsborrown, P. (2013), "Cellulosic ethanol production from agricultural residues in Nigeria", *Energy Policy*, **63**, 207-214.
Julián, C.F.T., Quintero, A., Agudelo, R.A, Cardona, C.A. and Higuita, J.C. (2011), "Analysis of coffee cut-stems (CSS) as raw material for fuel ethanol production", *Energy*, **36**, 4182-4190.
Kheiralla, A.F., El-Awad, M.M., Hassan, M.Y., Hussen, M.A. and Hind, I. (2012), "Experimental determination of fuel properties of ethanol/gasoline blends as bio-fuel for SI engines", *International Conference on Mechanical, Automobile and Robotics Engineering (ICMAR'2012)*, Penang, Malaysi, July.
Koçar, G. and Civaş, N. (2013), "An overview of biofuels from energy crops: current status and future prospects", *Renew. Sustain. Energy Rev.*, **28**, 900-916.
Ko, C.H., Wanga, Y.N., Chang, F.C., Chen, J.J., Chen, W.H. and Hwange, W.S. (2012), "Potentials of lignocellulosic bioethanols produced from hardwood in Taiwan", *Energy*, **44**, 329-324.
Kim, T.H. (2014), "Overview of technical barriers and implementation of cellulosic ethanol in the U.S", *Energy*, **66**, 13-19.
Karuppaiya, M., Viruthagiri, T. and Manikandan, K. (2012), "Statistical screening of medium components on ethanol production from cashew apple juice using saccharomyces diasticus", *Int. J. Biolog. Veterin. Agricult. Food Eng.*, **6**, 64-67.
Larissa, C.B.A.B., Fabio, R.M.B., Ferreira, E.A.C. and Meirelles, A.J.A. (2013), "Performance and cost evaluation of a new double-effect integration of multicomponent bioethanol distillation", *Energy*, **63**, 1-9.
Larissa, C.B.A.B., Fabio, R.M.B. and Antonio, J.A.M. (2012), "Double-effect integration of multicomponent alcoholic distillation columns", *Energy*, **45**, 603-612.
Limtong, S., Sringiew, C. and Yongmanitchai, W. (2007), "Production of fuel ethanol at high temperature from sugar cane juice by a newly solated Kluyveromycesmarxianus", *Biores. Technol*., **8**, 3367-3374.
Li, Y., Horsman, M., Wu, N., Lan, C.Q. and Dubois-Calero, N. (2008), "Biofuels from microalgae", *Biotechnol. Prog.*, **24**, 815-820.
Musa, U. (2012), "Extraction and transesterification of Jatropha caucus oil for biodiesel production", M.Eng. Thesis, Federal University of Technology, Minna.
Maity, J.P., Hou, C.P., Majumder, D., Bundschuh, J., Kulp, T.R., Chen, C.Y., Chuang, L.T., Chen, C.N.N., Jean, J., Ojeda, T.K., Sánchez, E. and Kafarov, V. (2011), "Sustainable ethanol production from lignocellulosic biomass - application of exergy analysis", *Energy*, **36**, 2119-2128.
Pimpakan, P., Yongmanitchai, W. and Limtong, S. (2012), "Bioethanol production from sugar cane syrup by thermo-tolerant yeast, Kluyveromyces marxianus DMKU3-1042, using fed-batch and repeated-batch fermentation in a nonsterile system", *Kasetsart J. Nat. Sci.*, **46**, 582-591.
Popov, S., Ronkovic, J., Dodic, J., Dodic, S. and Jokic, A. (2010), "Bioethanol production from raw juice as intermediate of sugar beet processing. A RSM approach", *Food Technol. Biotechnol*., **48**, 376-383.
Quinteroa, J.A., Montoyaa, M.I., Sancheza, O.J., Giraldoc, O.H. and Cardonaa, C.A. (2008), "Fuel ethanol

production from sugarcane and corn: comparative analysis for a colombian case", *Energy*, **33**, 385-399.
Ranković, J., Dodić, J., Dodić, S., Popov, S., Jevtić-Mučibabić, R. (2009), "Thin juice from sugar beet processing as medium for bioethanol production", *Zbornik radova Tehnološkog fakulteta u Leskovcu*, **19**, 44-51.
Suleiman, V., Olawale, A.S. and Waziri, S.M. (2014a), "Exergetic economic assessment of distillation based hybrid configurations for bioethanol refining", *Int. J. Thermodyn.*, **17**(4), 221-231.
Suleiman, B., Abdulkareem, A.S., Eyikanmi, T.A. and Afolabi, E.A. (2014b)**,** "Statistical optimiozation of bioethanol produced from sugarcane juice", *7th International Conference on Sustainable Energy and Environmental Protection*, Dubai-UAE, November.
Silva, C.G. (2010), "Renewable energies: choosing the best options", *Energy*, **35**, 3179-3193.
Sadorsky, P. (2011), "Some future scenarios for renewable energy", *Features*, **43**, 1091-1104.
Sobrinho, V.S., Ferreira da Silva, V.C. and Cereda, M.P. (2011), "Fermentation of sugar cane juice (Sacharum officinarum) cultivar RB 7515 by wild yeasts resistant to UVC", *J. Biotechnol. Biodiver.*, **2**, 3-21.
Theuretzbachera, F., Bauera, A., Lizasoain, J., Beckerc, M., Rosenau, T., Potthast, A., Fried, A., Piringera, G. and Gronauera, A. (2013), "Potential of different Sorghum bicolor (L. moench) varieties for combined ethanol and biogas production in the pannonian climate of Austria", *Energy*, **55**, 107-113.
Taherzadeh, M.J. and Karimi, K. (2007), "Acid based hydrolysis processes for ethanol from lignocellulosic materials: a review", *Bioresources*, **2**(3), 472-499.
Walker, G.M. (2010), "Bioethanol: Science and Technology of fuel alcohol", University of Abertay Dundee, Scotland.
Zegada-Lizarazu, W. and Monti, A. (2012), "Review: are we ready to cultivate sweet sorghum as a bioenergy feedstock? A review on field management practices", *Biomass Bioenergy*, **40**, 1-12.

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