Journal of ENVIRONMENTAL Treatment Techniques

J. Environ. Treat. Tech.
ISSN: 2309-1185

Journal web link: http://www.jett.dormaj.com

Bioethanol Production from Cassava Mill Effluents Supplemented with Solid Agricultural Residues Using Bakers' Yeast (*Saccharomyces cerevisiae*)

Sylvester Chibueze Izah* and Elijah Ige Ohimain

Bioenergy and Environmental Biotechnology Research Unit, Biological Sciences Department, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Received: 24/01/2015 Accepted: 14/02/2015 Published: 30/03/2015

Abstract

Nigeria is the leading cassava and fifth largest oil palm producer, accounting for 54 and 0.93 million metric tonnes respectively. As such during processing large wastes are generated including chaff, palm press fiber, palm kernel shell, empty fruit bunch (EFB) and palm oil mill effluents from oil palm and cassava peels and cassava mill effluents (CME). These wastes are discharged into the ecosystem without treatment where they cause attendant environmental impacts. This study evaluated bioethanol production from CME supplemented with chaff, EFB and cassava peels through separate hydrolysis and fermentation (SHF) techniques. CME with initial pH of 3.6 were used for hydrolysis and results showed a total reducing sugar of 0.31±0.006 mg/ml (CME + EFB -Treatment A), 0.25±0.002 mg/ml (CME +Chaff - Treatment B), 0.43±0.007 mg/ml CME + Cassava peels - Treatment C) and 0.20±0.002mg/ml (CME i.e not hydrolyzed – Treatment D). Fermentation was carried out with Bakers' yeast (*Saccharomyces cerevisiae*) under candle jar and highest ethanol volume, weight, concentration, yield and fermentation efficiency was respectively observed as 54.00±0.289ml, 49.56±0.058g, 1.25±0.001%, 0.16 $Y_{p/s}$ and 31.34% at fermentation period of 120 hours (Treatment A), 92.00±0.2889ml, 87.42±0.557g, 0.94±0.010%, 0.35 $Y_{p/s}$ and 68.56% at incubation period of 72 hours (Treatment B), 66.00±0.404ml, 62.84±0.557g, 1.25±0.012%, 0.15 $Y_{p/s}$ and 28.65% at fermentation time of 120 hours (Treatment C), 54.00±0.153ml, 49.47±0.199g, 0.60±0.006%, 0.25 $Y_{p/s}$ and 48.55% at fermentation duration of 144 hours (Treatment D) was observed. The specific gravity of the ethanol produced from the various Treatment ranged from 0.8875 – 0.9673. The study concluded that CME supplemented with EFB, chaff and cassava peels could generate ethanol and thus can be used as a lignocellulosic ethanol feedstock.

Keywords: Agricultural processing waste, Bioethanol, Cassava, Environmental management, Nigeria, Oil palm

1 Introduction

Nigeria is a nation blessed with non-renewable energy resources such as petroleum and natural gas. Like conventional fossil energy resources, the country has abundance cheap renewable energy resources including biomass, solar, wind, hydropower etc. Over the years, petroleum and natural gas products have been the major sources of energy supply. About 90% and 85% of Nigeria export and earning respectively are provided by petroleum [1, 2]. Petroleum also contributes significantly to the gross domestic products (GDP) of the country, seconded by cassava [3]. In 1980, oil accounted for 29% of Nigeria's GDP and rose to 52% in 2005. Generally, till date, no other primary energy source is flexible and cheap as petroleum [2]. Presently, fossil energy resources which provides about 50% of total energy used globally [4] and about 86% of total transportation fuel [5] is depleting [6, 7] due to increased population and economic growth [8]. Like other advanced nations in the world, Nigeria has joined the host of countries in search of alternative energy resources. Renewable energy sources provide several solutions to the challenge of conventional energy resources such as emission of pollutant gases into the atmosphere.

Biomass is one of the essential renewable energy sources currently being utilized in the field of bioenergy due to its environmental compatibility and sustainability [9]. Biomass have been extensively exploited for solid fuel (Biochar, briquette), liquid fuel (biooil, bioethanol, biodiesel, biomethanol, biobutanol, and gaseous fuel (biogas and biohydrogen). Most of the feedstocks that have been exploited for biofuel are first and second generation feedstocks. The first generation biofuel is challenged by food versus fuel crises which could cause severe starvation in the world, if these feedstocks are only used for the production of biofuel. Besides, first generation feedstocks has high production cost [10]. These feedstocks are mostly grains, carbohydrates, vegetable oil etc. The production of biofuels from second generational feedstocks could also put land use under stress. Sameera *et al*. [11] classified

Corresponding author: Sylvester Chibueze Izah, Bioenergy and Environmental Biotechnology Research Unit, Biological Sciences Department, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. E-mail: sylvesterizah@yahoo.com: Tel: +2347030192 466

bioalcohols, biodiesel, green diesel, vegetable oil, bioethers, biogas, syngas, solid biofuels as first generational biofuels; Algae fuel, cellulosic ethanol, biohydrogen, biomethanol, 2,5-Dimethylfuran (DMF), Dimethyl ether (BioDME), Fischer-Tropsch (FT) diesel, biohydrogen diesel, mixed alcohols and wood diesel as second generation biofuels; and direct cellulose or lignocellulosic fermentation as third generational biofuel. Ohimain [12] reported that Fisher-Tropsch fuel such as FT diesel, FT gasoline and green chemicals can be produced from feedstocks found in Nigeria.

Bioethanol is an example of liquid biofuel produced from renewable resources [13] through fermentation [14]. Bioethanol via blending can be used to substitute petrol or gasoline used in combustion engines. Nigeria biofuel policy have approved 10% ethanol blend called gasohol [1, 2, 12, 14 – 16]. Flexible fuel vehicle have also been produced and can run up to 85% ethanol and 15% gasoline [14]. However, 10% ethanol blend is the most common [17]. The use of bioethanol as fuel proffer several advantages including high heat of vaporization, compression ratio and power generation [18]. Biofuel is basically non-flammable, non-toxic, biodegradable [6, 11], available, and potential employment contributor. Bioethanol production could enhance the living conditions of the people involved in different stage of operation. Bioethanol can promote energy and environmental aims [19] such as reduction in net carbon dioxide emission to the atmosphere [12, 17, 20, 21].

In Nigeria most of the feedstocks currently considered for bioethanol production are sugar cane, cassava, sweet sorghum [16]. Other bioethanol producing nations depend on sugarcane (Brazil), sugar beets (Europe), cassava (China, Thailand), sorghum (India, Philippines), Corn (USA). The production of bioethanol from these feedstocks could cause adverse effects due to its edibility. These challenges could be reduced through the use of non-edible grasses and wastes cellulosic/ lignocellulosic biomass such as switch grass (*Panicum virgatum), Miscanthus sp*. giant reed (*Arundo donax*) and reed canary grass (*Phalaris arundinacea)* [22], elephant grass (*Pennisetum purpureum*) [23, 24], wild sorghum etc. and lignocellulosic waste feedstocks.

Lignocellulosic material from agricultural crop residue appears to be a suitable option for bioethanol production. Several agricultural biowastes have been widely unutilized in Nigeria [12]. These wastes typically causes environmental nuisance. For instance, biowastes from oil palm and cassava processing mills in the form of solid and liquid causes attendant environmental pollution due to the unsustainable wastes disposal system being practiced by the processors.

During ethanol conversion several microorganisms have been employed such as *Bacillus carotarum*, *Bacillus lentus*, *Bacillus stearothermophilus*, *Bacillus pumilis*, *Micrococcus luteus*, *Yarrowia lipolytica*, *Candida intermedia*, *Candida tropicalis*, *Clavispora lusitaneae*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceous* and *Penicillium* species, *Zymomonas mobilis* [21, 25 – 29]. Generally, *Saccharomyces cerevisiae* and *Zymomonas mobilis* have been recommended for use for ethanol production due to the advantages that they confer. Modified microbes such as baker's yeast and EMCE-ferm [14], brewer's yeast [20] have been widely employed. Generally, conventional yeast can convert glucose to ethanol but cannot ferment xylose [14].

Nigeria is the fifth largest producer of oil palm [30] and the leading producer of cassava in the world [31 – 39]. Despite the potentials of bioethanol productions from oil palm and cassava processing wastes such as chaff, empty fruit bunch (EFB), cassava peels (Figure 1), cassava mill effluents (CME). The use of CME for the hydrolysis of these solid wastes is scanty in literatures. Therefore this study aimed at comparing ethanol production from CME supplemented with EFB, chaff and cassava peels.

Empty fruit bunch (EFB) Chaff Cassava peels

Figure 1: Oil palm and cassava processing solid wastes used in the study

2 Materials and Methods

2.1 Field Sampling

A cassava processing mill at Otuogori in Ogbia Local Government Area, Bayelsa State, Nigeria was visited 30 October to 02 November 2014. Triplicate samples of CME and cassava peels produced during cassava peeling prior to milling and CME generated after milling/ pressing were collected with a jute bag and wide mouth container respectively. Similarly, a field trip was undertaken on 20th October to 22nd October to oil palm processing mills at Elele in Emuoha Local Government Area of Rivers State, Nigeria and triplicate samples of oil palm processing chaff and empty fruit bunch generated prior to sieving were collected with jute bag. The baker's yeast (*Saccharomyces cerevisiae*) used in this study was obtained from Tombia

market in Yenagoa Local Government Area, Bayelsa State, Nigeria.

2.2 Sample preparations

The solid samples such as EFB, Chaff and cassava peelings was sun dried and milled to powered form using hammer mills. The CME were allowed to stand for 48 hours and it was decanted i.e separating the supernatant at the bottom from the clear less turbid effluents at the top.

2.3 Experimental design

Completely Randomized Design (CRD) experimental approach was used for this study. The use of the CRD experimental design was based on the fact that the feedstock (CME) used in the study was homogenous. The CME used were randomly divided into four groups including one control group i.e Treatment (A, B, C and D). The various groups have initial pH value of 3.6. Treatment A, B and C were supplemented with EFB, chaff, and cassava peels respectively, while Group D served as the control Treatment and have no supplement

2.4 pH meter calibration

The pH was determined in–situ by the method described by Ademoroti [40] using pH meter (HANNA HI 9820). The pH electrode was calibrated at pH 4, 7 and 10 with pH buffers prior to use.

2.5 Bioethanol production

During bioethanol production, hydrolysis, fermentation and distillation were independently carried out. Figure 2 presents the processes employed for the ethanol production in this study.

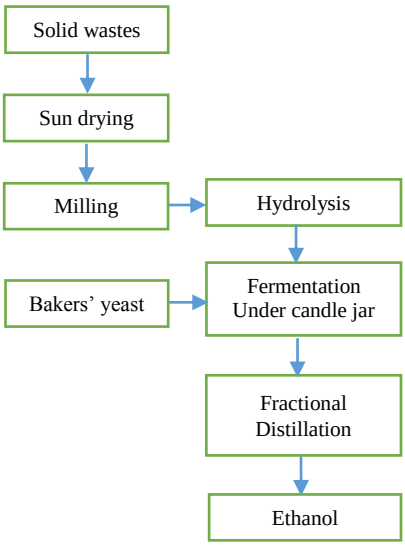

Figure 2: Flow chart of ethanol production

2.5.1 Hydrolysis

One hundred gram (100g) of the grounded samples was weight into different cornical flask and CME were added to the cornical flask to bringing the volume to 1000 ml. The flask was covered with cotton wool wrapped in aluminum foil [14, 17]. The flask was heated at a temperature of about 100ºC for 1 hour in a loosely closed autoclave before closing properly and autoclave for 15 minutes at 121°C. The flasks containing the different samples were allowed to cool before filtering with a mesh. The resultant filtrates were filtered with White man filter paper. The pHs of the hydrolyzed Treatments are 3.7 (A) 3.9 (B) and 3.8 (C) and Treatment D was not hydrolyzed and has initial pH of 3.6.

2.5.2 Fermentation

The samples containing the various group was covered air tight and bring to boiling at a temperature of about 100ºC. Then, the samples was transferred to a laminar flow and kept under ultra violet light for 1 hour. About 1g of bakers' yeast (*Saccharomyces cerevisiae*) was aseptically transferred into the containers containing the various Treatments and the lid was covered. The flask was incubated at room temperature for 7 days with each sample from the various Treatment, (A – D) taken for 24 hours up to 7 days for distillation.

2.5.3 Fractional distillation

The fermented samples were transferred into round bottom flask fixed to a distillation column with running tap water for cooling [14, 17, 21]. A cornical flask was fixed to a distillation column at the other end to collect the distillate. The temperature of the distillatory heating mantle was adjusted to 78°C and this was maintained throughout the experiment [14, 17, 21, 25].

2.6 Determination of ethanol yield and properties

2.6.1 Density and specific gravity

Density was determined using the scheme of Ademiuyi and Mepba [41]. The volume and mass of the distillates were determined with measuring cylinder and weighing balance respectively. The density and specific gravity was calculated using the formula:

$$Density\ \left(\frac{g}{ml}\right) = \frac{Mass}{Volume} \quad (1)$$

$$Specific\ fravity = \frac{Density\ of\ ethanol}{Density\ of\ water} \quad (2)$$

2.6.2 Solubility

The method described by Garba *et al*. [42] was used. The distillate at 78ºC was mixed with distilled water and shaken. No distinct layer indicates that the sample is miscible with water.

2.6.3 Appearance

The appearance of the distillates was based on physical observations.

2.6.4 Determination of total reducing sugar and ethanol concentration

The concentration of total reducing sugar (TRS) was determined colorimetrically using 3, 6-dinitrosalicyclic acid (DNS) method. About 1 ml of filtrate was mixed with 1 ml of DNS and heated at 100 ºC for 5 minutes. The reaction was stopped and incubated on ice. The optical density was determined at 540nm from spectrophotometer (Perklin Elmer EZ 301). The concentration of residual

sugar was determined against a glucose standard graph [42]. Gas chromatography was used in the determination of ethanol concentration using the method described by Konlani *et al*. [43].

2.6.5 Ethanol product analysis

The method previously described by Kassim *et al*. [27] was used for product analysis. Also fermentation efficiency described by Prasetyo *et al*. [44] and ethanol yield by Abengunde *et al.* [45] were employed. The ethanol yield ($Y_{p/s}$) was calculated as the actual ethanol produced as gram ethanol per gram of total sugar consumed and the fermentation efficiency (%) was based on the ratio of ethanol (g/l) to the theoretical maximum yield. The mathematical formulas are presented in equation 3 – 4.

$$\text{Fermentation efficiency } (\%) = \frac{\text{Ethanol g/l x 100}}{\text{Glucose, g/l x 0.51}} \quad (3)$$

$$\text{Ethanol yield (Y p/s)} = \frac{Ethanol, \text{g/l}}{\text{Glucose, g/l}} \quad (4)$$

2.6 Statistical Analysis

SPSS software version 16 was used to carry out the statistical analysis. Descriptive statistics were used to show the mean and standard error. A one-way analysis of variance was carried out at p = 0.05, and Duncan's multiple range test was used to discern the source of the observed differences. The chart were plotted with Microsoft excel package 2013.

3 Results and Discussion

The concentration of the total reducing sugar prior to fermentation from the various feedstocks is presented in Figure 3. The concentration of total reducing sugar was 0.31±0.006 mg/ml (Treatment A), 0.25±0.002 mg/ml (Treatment B), 0.43±0.007 mg/ml (Treatment C) and 0.20±0.002mg/ml (Treatment D). The highest and least concentration was observed in Treatment C and D respectively. However, there were significant difference among the various Treatments ($P< 0.05$). During hydrolysis, the reducing sugar found in the feedstock was degraded by the action of acidity of the CME. Based on the study, CME supplemented with cassava peels had the highest sugar concentration. All the substrate contains glucose, but the treatment with supplement higher sugar content. The order of sugar in the Treatments are C>A>B >D. The concentration of glucose reported in this study is similar to previous reports from food processing wastes. Itelima *et al*. [26] reported reducing sugar from banana, plantain and pineapple peels as 0.20 – 0.82 mg/cm^3, 0.16 - 0.45 mg/cm^3 and 0.27 – 0.94 mg/cm^3 respectively. Similarly, the findings of this study have higher sugar content from study from CME feedstocks by authors. Glucose concentrations of 1.37 mg/g [25] and 5.62 mg/l [20] have been reported from CME. Basically glucose is the most abundant sugar found in lignocellulosic feedstocks.

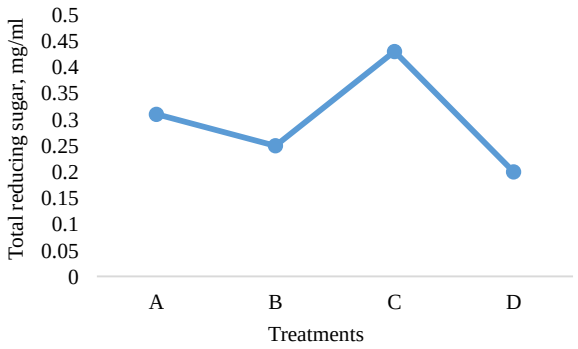

Figure 3: Concentration of total reducing sugar prior to fermentation from the various feedstocks

The volume of ethanol produced from the various Treatments is presented in Table 1. The highest volume occurred at 120 hours of fermentation (54.00±0.289 ml) for Treatment A, 72 hours of fermentation (92.00±0.2889 ml) for Treatment B, 120 hours of fermentation (66.00±0.404 ml) for Treatment C and 144 hours of fermentation (54.00±0.153 ml) for Treatment D. At these fermentation periods, the volume of the ethanol produced began to decrease. There were significant differences ($P< 0.05$) in the volume of ethanol in the various Treatment group in each of the fermentation duration. The volume of ethanol produced in the various treatment was in the order of Treatment B > Treatment C > Treatment D > Treatment A. The volume of ethanol produced is comparable to previous study. Prasad *et al*. [13] studied bioethanol production from several agricultural raw materials by two step enzymatic process using *Saccharomyces cerevisiae* and reported volume of bioethanol produced out of 250 ml of extract before and after distillation respectively as 221 and 29 ml (potato), 216 and 34 ml (sweet potatoes), 219 and 31 ml (cassava), 211 and 39 (fruit extract), 226 and 24ml (boiled rice water), 233 and 17 ml (rice husk), 238 and 12 ml (rice straws), 241 and 9 ml (wood bark), 203 and 47 ml (sugar cane beets), 232 and 18 ml (waste paper), 239 and 11 ml (saw dust), 245 and 5 ml (coconut pitch), 231 and 19 ml (groundnut waste), 243 and 7 ml (leaf litter) and 231 and 19 (maize husk).

Table 2 presents the weight of ethanol produced from the various Treatments. In Treatment A, the highest weight occurred at 120 hours of fermentation (49.56±0.058 g). In Treatment B, weight of 85.99±0.026 g was achieved at 72 hours of fermentation. In Treatment C, weight of 62.84±0.577 g was achieved at 120 hours of fermentation and in Treatment D, weight of 49.47±0.199 g was achieved at 144 hours of fermentation. The weight of the ethanol produced increased from 24 hours before reaching the optimum fermentation hour and then decreased. There was significant difference ($P< 0.05$) in the various Treatment group in each of the fermentation periods. The weight of the ethanol produced followed the trend of the volume generated during distillation.

Table 1: Volume of ethanol produced (ml) from the different feedstocks

Fermentation period, hours	Treatment			
	A	B	C	D
24	10.00±0.058b	51.00±0.115c	38.27±0.481d	17.00±0.173a
48	14.00±0.231b	73.00±0.115c	36.00±0.115d	22.00±0.289a
72	28.00±0.577b	92.00±0.289b	48.00±0.231c	40.00±0.265a
96	42.00±0.173c	90.00±0.265a	58.00±0.256b	42.00±0.208d
120	54.00±0.289c	86.00±0.173b	66.00±0.404a	50.07±0.240a
144	36.00±0.404b	80.33±1.967a	56.00±0.252b	54.00±0.153a
168	24.00±0.577b	64.07±0.233a	49.00±0.513c	52.00±0.252a

Each value is expressed as mean ± standard error (n = 3). Different letters in each row indicate significant differences at $P< 0.05$ according to the Duncan Statistics.

Table 2: Weight of ethanol produced (g) from the different feedstocks

Fermentation period, hours	Treatment			
	A	B	C	D
24	8.88±0.060a	48.40±0.006d	34.86±0.035c	22.78±0.023b
48	12.43±0.029a	69.27±0.023d	33.03±0.017c	29.48±0.277b
72	24.85±0.577a	87.42±0.577d	44.04±0.554c	38.23±0.028b
96	38.73±0.017a	85.99±0.026d	53.58±0.012c	39.08±0.006b
120	49.56±0.058b	81.61±0.577d	62.84±0.577c	46.71±0.410a
144	31.95±0.015a	76.05±0.050d	54.17±0.030c	49.47±0.199b
168	21.30±0.577a	60.84±0.040d	47.27±0.291b	49.40±0.021c

Each value is expressed as mean ± standard error (n = 3). Different letters in each row indicate significant differences at $P< 0.05$ according to the Duncan Statistics.

Figure 4 presents the density/ specific gravity of the ethanol produced from the various Treatments. The concentration ranged from 0.8875 - 0.9673 g/ml across all the treatments. The specify gravity of the ethanol produced from this study is close to the findings of other authors. Janani *et al*. [46] reported specific gravity of ethanol produced from different fruit wastes as 0.860 at 48hrs (grape), 0.885 at 36 hours (apple), 0.872 at 72 hours (papaya) and 0.834 at 72 hours (banana). Basically, the decrease in specific gravity value is an indication of yeast fermenting the sugar leading to ethanol production, hence specific gravity is a measure of sugar content of a substrate [46]. The specific gravity from this study is comparable to report an author from different feedstocks. Prasad *et al*. [13] reported specific gravity of 0.9645 (potato), 0.9429 (sweet potatoes), 0.9441 (cassava), 0.9321 (fruit extract), 0.9598 (boiled rice water), 0.9426 (rice husk), 0.9557 (rice straws), 0.9692 (wood bark), 0.8418 (sugar cane beets), 0.9817 (waste paper), 0.9231 (saw dust), 0.9736 (coconut pitch), 0.9441 (groundnut waste), 0.9121 (leaf litter) and 0.9724 (maize husk). Generally, the specific gravity of ethanol produced from this study is higher than the absolute specific gravity of ethanol (0.79) [13]. The concentration of ethanol produced from the various feedstocks is presented in Table 3. The highest concentration of ethanol for Treatment A (1.25±0.001%) was achieved at 120 hours of fermentation; in Treatment B, concentration of 0.94±0.010 % was achieved after 72 hours of fermentation; in Treatment C, concentration of 1.25±0.012 % were achieved at 120 hours of fermentation; while in Treatment D, highest concentration of 0.60±0.006% was achieved at 144 hours of fermentation. There was significant difference ($P< 0.05$) in the ethanol concentrations among the various treatments in each of the fermentation periods. Fermentation efficiency of the feedstocks from the various treatment categories is presented in Figure 5.

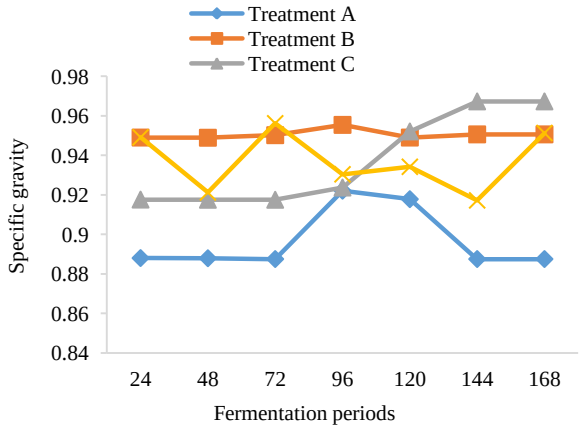

Figure 4: Density (g/ml)/specific gravity of the ethanol produced from the various Treatments

The highest fermentation efficiency of 31.34% was achieved at 120 hours of incubation (Treatment A), 68.56% was achieved at 72 hours of fermentation (Treatment B), 28.65% was achieved at 120 hours of fermentation (Treatment C) and 48.55% was achieved at 144 hours of fermentation. Figure 6 presents the ethanol yield ($Y_{p/s}$) of the feedstocks from the various Treatment categories. Highest ethanol yield of 0.16$Y_{p/s}$ was achieved at 120 hours of incubation (Treatment A), 0.35$Y_{p/s}$ was achieved

at 72 hours of fermentation (Treatment B), 0.15 was achieved at 120 hours of fermentation (Treatment C) and 0.25 was achieved at 144 hours of incubation (Treatment D).

Table 3: Ethanol concentration (%) from the feedstocks under study

Fermentation period, hours	Treatment			
	A	B	C	D
24	0.42±0.012a	0.53±0.017d	0.92±0.010c	0.28±0.015b
48	0.60±0.017a	0.71±0.012d	0.82±0.010c	0.33±0.017b
72	0.95±0.006a	0.94±0.010d	1.05±0.012c	0.38±0.012b
96	1.20±0.002a	0.81±0.019d	1.11±0.010c	0.43±0.012b
120	1.25±0.001b	0.74±0.010d	1.25±0.012c	0.46±0.010a
144	1.10±0.006a	0.61±0.006d	1.09±0.035c	0.60±0.006b
168	0.86±0.012a	0.53±0.015d	1.00±0.000b	0.53±0.015c

Each value is expressed as mean ± standard error (n = 3). Different letters in each row indicate significant differences at $P< 0.05$ according to the Duncan Statistics.

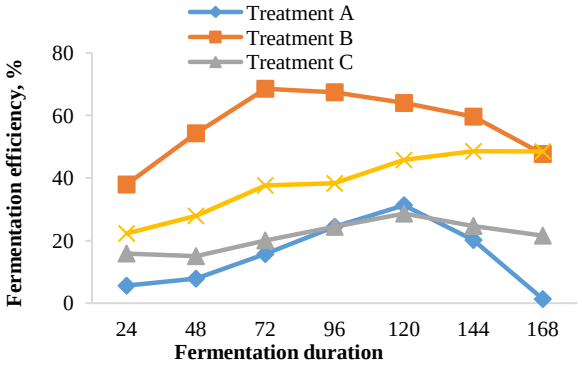

Figure 5: Fermentation efficiency (%) of ethanol production from the various Treatments

From this study, it was found that bioethanol yield from the various hydrolysate was 0.16g ethanol/g glucose at 120 hours of incubation (Treatment A), 0.35g ethanol/g glucose at 72 hours of incubation (Treatment B), 0.15g ethanol/g glucose at 120 hours of incubation (Treatment C) and 0.25g ethanol/g glucose at 144 hours of incubation (Treatment A).

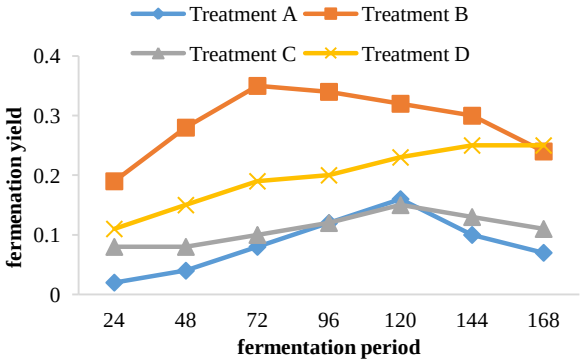

Figure 6: Ethanol yield ($Y_{p/s}$) produced from the various Treatments

The various period optimum ethanol yield was obtained could be due to the fact that *Saccharomyces cerevisiae* used in the study has reached its optimum growth density [42] in the various incubation period for the different Treatment groups. Kassim *et al*. [27] reported ethanol production from EFB hydrolysate to be 0.51g ethanol/g glucose at 72 hours of incubation. During these processes, glucose in the substrates are broken down by the organisms to form pyruvate via the glycolytic pathway, which resulted in the production of ethanol under anaerobic conditions [47]. Ethanol yield using *Saccharomyces cerevisiae* increased initially after inoculation and declined at the end of the fermentation period in all the Treatments. The concentration of the ethanol produced were in the order of Treatment C = Treatment A> Treatment B > Treatment D. Similarly, the highest ethanol concentration were achieved after hours of fermentation in the order of Treatment B (at 96 hours) < Treatment C = Treatment A (at 120 hours) < Treatment D (at 144 hours). The superiority of ethanol concentration by Treatment A and C is an indication of superior glucose content. The amount of ethanol content increased with an increase in fermentation time [48], before finally declined. Treatment B had the highest ethanol volume, weight, yield and fermentation efficiency. This could be attributed to better pH (3.9) prior to fermentation and/ or less potential inhibitory materials. The pH of the CME used for hydrolysis and final prior to fermentation was <4. This is within the range of 3.5 – 6.0 reported to ferment substrate efficiently by yeast [10]. Beside pH, Pramanik [49] has reported that formation of undesirable products such as glycerol and organic acid could lead to lower ethanol yield. This could have significantly affected Treatment A and C. Similarly, Garba *et al*. [42] noted that low ethanol yield after 5 day of fermentation of substrate is an indication that the hydrolyzate contain large quantity of metabolic inhibitor such as Furfural, which can interfere with fermentation processes. In the respective feedstocks of this study, the inhibitory materials they contain might be different; hence there were relative variation in the yield. Again, the composition of the yeast could have significantly affected the ethanol formation. Nzelibe and Okafoagu [14] stated that conventional yeast can only ferment glucose and not xylose, which consist of 30% of sugar in the residues. Generally, the ethanol yield from this study is comparable to the yield of $0.26Y_{p/s}$ (from SSF batch without detoxification) and 0.37 $Y_{p/s}$ (from SSF fed batch with detoxification) from cassava peels as reported by Kongkiattikajorn [50].

Table 4 presents the appearance and solubility of ethanol produced from the different feedstocks. On

appearance, the bioethanol produced in all the various treatments was colorless and highly soluble in water. The result of this study is comparable to the report of Ademiluyi and Mepba [41] and Walker [51] on the colour of ethanol and report of Garba *et al*. [42] and Walker [51] on the solubility of ethanol in distilled water.

Table 4: Solubility and appearance of the ethanol produced from the different feedstocks

Parameters	Treatment			
	A	B	C	D
Solubility	Highly miscible	Highly miscible	Highly miscible	Highly miscible
Appearance	Colorless	Colorless	Colorless	Colorless

4 Conclusion

This study aimed at investigating bioethanol production from CME supplemented with EFB and chaff from oil palm and cassava peels. The volume of ethanol were highest in the order of Treatments B>C>D>A. The concentration of ethanol were higher with the Treatment that contain supplement (i.e Treatment A-C) when compared to the control sample (Treatment D). Hence, ethanol production from CME could give an improved result with addition of other lignocellulosic feedstock produced from food processing. The conversion of agricultural residues mainly from oil palm (i.e empty fruit bunch and chaff) and cassava processing wastes (such as cassava peels and CME) to bioethanol can be efficient, economical, useful, and practical approach of managing food processing wastes. Hence, the conversion of this waste could contribute significantly to the Nigeria biofuel industry if all these wastes generated are collected and channeled into bioethanol production. It could also create employment and reduce importation of bioethanol to meet the 10% ethanol blend (E10) approved by the Nigeria automotive industry for incorporation into gasoline consumed in the country.

Acknowledgement

This study was based of Master of Science project work of Sylvester Chibueze Izah supervised by Dr. Elijah Ige Ohimain at the Niger Delta University, Nigeria.

References

1- Ohimain, E.I. (2013). Can the Nigerian biofuel policy and incentives (2007) transform Nigeria into a biofuel economy? *Energy Policy*, 54, 352–359.
2- Ohimain, E.I. (2013). The challenge of liquid transportation fuels in Nigeria and the emergence of the Nigerian automotive biofuel programme. *Research Journal of Applied Sciences, Engineering and Technology*, 5(16), 4058-4065.
3- Jackson, B.A., Oladipo, N.O., & Agaja, M.O. (2013). Cassava: A Potential Crop for Industrial Raw Material in Nigeria. *International Journal of Life Sciences*, 3(3), 105-112.
4- Ohimain, E.I. (2010). Petroleum Geomicrobiology. In: Jain SK, Khan AA and Rain MK (Editors). *Geomicrobiology: Biodiversity and Biotechnology*. CRC Press/Taylor and Francis, Boca Raton, Florida, USA. Pp. 139 – 174
5- Atadashi, I.M., Aroua, M.K., & Aziz, A.A. (2011). Biodiesel separation and purification: a review. *Renewable Energy*, 36, 437 – 443.
6- Izah, S.C., & Ohimain, E.I. (2013). The challenge of biodiesel production from oil palm feedstock's in Nigeria. *Greener Journal of Biological Sciences*, 3(1), 1 - 12.
7- Tiwari, S., Jadhav, S.K., Sharma, M., & Tiwari, K.L. (2014). Fermentation of waste fruits for bioethanol product. *Asian Journal of Biological Sciences*, 7(1), 30 – 34.
8- Daud, M., Syafii, W., & Syamsu, K. (2012). Bioethanol production from several tropical wood species using simultaneous saccharification and fermentation processes. *Wood Research Journal*, 3(2), 106 – 115.
9- Barz, M., & Delivand, M.K. (2011). Agricultural residues as promising biofuels for biomass power generation in Thailand. *Journal of Sustainable Energy and Environment Special Issue*, no volume, 21– 27.
10- Alam, M.Z., Kabbashi, N.A., & Hussin, S.N.I.S. (2009). Production of bioethanol by direct bioconversion of oil palm industrial effluents in a stirred tank bioreactor. *Journal of Industrial Microbiology and Biotechnology*, 36, 801 – 808.
11- Sameera, V., Sameera, C., & Ravi, T.Y. (2011). Current strategies involved in biofuel production from plants and algae. *Journal Microbial and Biochemical Technology*, R1:002. Doi:10.4172/1948-5948.R1-002.
12- Ohimain, E.I. (2013). A review of the Nigerian biofuel policy and incentives (2007). *Renewable and Sustainable Energy Reviews*, 22, 246–256.
13- Prasad, M.P., Rekha, S., Tamilarasan, M., & Subha, K.S. (2009). Production of Bioethanol using various agricultural raw materials by two step enzymatic process. *Advanced Biotech*, no volume, 41 – 43.
14- Nzelibe, H.C.N., & Okafoagu UC (2007). Optimization of ethanol production from garcinia kola (bitter kola) pulp Agro Waste. *African Journal of Biotechnology*, 6(17), 2033-2037.
15- Agboola, O.P., Agboola, O.M., & Egelioglu, F. (2011). Bio-ethanol derivation from energy crop in Nigeria: a path to food scarcity or bio-fuel advancement. Proceedings of the World Congress on Engineering, July 6 - 8, 2011, London, U.K.
16- Ohimain, E.I. (2010). Emerging bio-ethanol projects in Nigeria: Their opportunities and challenges. *Energy Policy*, 38, 7161-7168.
17- Oyeleke, S.B., & Jibrin, N.M. (2009). Production of ethanol from guinea cornhusk and millet husk. *African Journal of Microbiology Research*, 3(4), 147 – 152.
18- Geller, H.S. (1985). Ethanol fuel from sugar cane in Brazil. *Annual Review in Energy*, 10, 135 – 164.
19- Farrell, A.E., Plevin, R.J., Turner, B.T., Jones, A.D., O' Hare, M., & Kammen, D.M. (2006). Ethanol can contribute to energy and environmental goals. *Science*, 311 (506), 1 -23.
20- Akponah, E., & Akpomie, O.O. (2012). Optimization of bio-ethanol production from cassava effluent using *Saccharomyces cerevisiae*. *African Journal of Biotechnology*, 11(32), 8110-8116.
21- Rabah, A.B., Oyeleke, S.B., Manga, S.B., & Hassan, L.G. (2011). Utilization of millet and guinea corn husks for bioethanol production. *African Journal of Microbiology Research*, 5(31), 5721-5724.
22- Lewandowski, I., Scurlock, J.M.O., lindvall, E., & Christou, M. (2003). The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy*, 25, 335 – 361.

23- Ohimain, E.I. (2013). The potentials of elephant grass (*Pennisetum purpureum* Schumach) bagasse as fuel for power generation. Third Electrical Engineering National Conference tagged "Energy Sources for Power Generation" (ES4PG-2013) holding from July 21-23, 2013 at the Department of Electrical Engineering, University of Nigeria, Nsukka.

24- Ohimain, E.I., Kenabie, P., & Nwachukwu, R.E.S. (2014). Bioenergy Potentials of Elephant Grass, *Pennisetum purpureum* Schumach. *Annual Research & Review in Biology*, 4(13), 2215 -2227.

25- Akponah, E., Akpomie, O.O., & Ubogu, M. (2013). Bio-ethanol production from cassava effluent using *Zymomonas mobilis and Saccharomyces cerevisiae* isolated from rafia palm (*Elaesis guineesis*) SAP. *European Journal of Experimental Biology*, 3(4), 247-253.

26- Itelima, J., Onwuliri, F., Onwuliri, E., Onyimba, I., & Oforji, S. (2013). Bio-ethanol production from banana, plantain and pineapple peels by simultaneous saccharification and fermentation process. *International Journal of Environmental Science and Development*, 4(2), 213 – 216.

27- Kassim, M.A., Kheang, L.S., Bakar, N.A., Aziz, A.A., & Som, R.M. (2011). Bioethanol production from enzymatically saccharified empty fruit bunches hydrolysate using *Saccharomyces cerevisiae*. *Research Journal of Environmental Sciences*, 5(6), 573-586.

28- Wakil, S.M., Fasiku, S.A., Adelabu, A.B., & Onilude, A.A. (2013). Production of bioethanol from spontaneous fermentation of palm oil mill effluent (POME). *Researcher*, 5 (2), 28-35.

29- Wakil, S.M., Fasiku, S.A., Adelabu, A.B., & Onilude, A.A. (2013). Production of bioethanol from palm oil mill effluent using starter cultures. *New York Science Journal*, 6(3), 77-85.

30- Izah, S.C., Oduah, A.A., & Ohimain, E.I. (2014). Effects of temperature and fermentation period on the recovery of second grade palm oil from palm press fiber. *International Journal of Engineering Science and Innovative Technology*, 3(5),131-138

31- Agwu, N.M., Nwachukwu, I.N., & Anyanwu, C.I. (2012). Climate variability: relative effect on Nigeria's cassava productive capacity. *Report and Opinion*, 4(12), 11-14.

32- Iheke, O.R. (2008). Technical efficiency of cassava farmers in South Eastern *Nigeria. Agricultural Journal*, 3(2), 152-156.

33- Karim, O.R., Fasasi, O.S., & Oyeyinka, S.A. (2009). Gari yield and chemical composition of cassava roots stored using traditional methods. *Pakistan Journal of Nutrition*, 8 (12), 1830-1833.

34- Muhammad-lawal, A., Salau, S.A., & Ajayi, S.A. (2012). Economics of improved and local varieties of cassava among farmers in Oyo state, Nigeria. *Ethiopian Journal of Environmental Studies and Management*, 5(2), 189 – 194.

35- Obisesan, A.A. (2012). Cassava marketing and rural poverty among smallholder farmers in southwest, Nigeria. *Bulletin of Environment, Pharmacology and Life Sciences*, 1 (8), 29 – 34.

36- Ohimain, E.I. (2013). Environmental impacts of smallholder ethanol production from cassava feedstock for the replacement of kerosene household cooking fuel in Nigeria. *Energy Sources Part A: Recovery, Utilization and Environmental Effects*, 35, 1-6.

37- Ohimain, E.I. (2013). Energy analysis of small-scale ethanol production from cassava: a case study of the cassakero project in Nigeria. *Journal of Technology Innovations in Renewable Energy*, 2, 119 – 129.

38- Ohimain, E.I., Silas-Olu, D. I. & Zipamoh, J.T. (2013). Biowastes generation by small scale cassava processing centres in Wilberforce Island, Bayelsa State, Nigeria. *Greener Journal of Environmental Management and Public Safety*, 2 (1), 51 – 59.

39- Okereke, C.O. (2012). Socio-economic evaluation of cassava production by women farmers in Igbo-Eze North Local Government Area of Enugu State, Nigeria. *International Journal of Agricultural Science, Research and Technology*, 2(3), 129-136.

40- Ademoroti, C.M.A. (2006). *Standard Method for Water and Effluents Analysis.* 1st Edition. Foludex press limited, Ibadan, Nigeria.

41- Ademiluyi, F.T., & Mepba, H.D. (2012). Yield and properties of ethanol biofuel produced from different whole cassava flours. *1SRN Biotechnology*, 2012, 1 – 6.

42- Garba, A., Salihu, G., Olatunde, A., Mohammad, S., Nasir, Z., Moses, E.E., & Bugaje, K.B. (2014). Production of bioethanol from millet stover by co-cultures of *saccharomyces cerevisiae* and *Aspergillus niger*. *International Research Journal of Scientific Findings*, 1 (2), 038-042.

43- Konlani, S., Delgenes, J.P., Moletta, R., Troare, A., & Doh, A. (1996). Optimization of cell yield of Candida Krusei SOI and Saccharomyces sp. LK3G cultured in sorghum hydrolysate. *Bioresource Technology*, 57, 275-281.

44- Prasetyo, J., Naruse, K., Kato, T., Boonchird, C., Harashima, S., & Park, E.Y. (2011). Bioconversion of paper sludge to biofuel by simultaneous saccharification and fermentation using cellulose of paper sludge origin and thermotorelant *Saccharomyces cerevisiae* TJ14. *Biotechnology for Biofuel*, 4:35. No page.

45- Abegunde, T.A., Oyewole, O.B., & Sanni, T.A. (2014). Ethanol yield of three varieties of cassava (Odongbo, Ofege, and TMS 30572) using α-amylase from germinated paddy rice and yeast from palm wine. *International Journal of Biological, Veterinary, Agricultural and Food Engineering*, 8(2), 171 – 175.

46- Janani, K., Ketzi, M., Megavathi, S., Vinothkumar, D., & Ramesh, B.N.G. (2013). Comparative Studies of Ethanol Production from Different Fruit Wastes UsingSaccharomyces cerevisiae. *International Journal of Innovative Research in Science, Journal Engineering and Technology*, 2(12), 7161 – 7167.

47- Shabna, A., Kale, R.D., & Nair, P. (2011).The potential bioconversion of paper waste into bioplastics and biofuel. *Biotechnology, Bioinformatics and Bioengineering*, 1(3), 337-342.

48- Rath, S., Singh, A.K., Masih, H., Kumar, Y., Peter, J.K., Mishra, P.S.S.K. (2014). Bioethanol production from waste potatoes as an environmental waste management and sustainable energy by using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae. International Journal of Advanced Research*, 2(4), 553-563 553.

49- Pramanik, K. (2003). Parametric studies on batch alcohol fermentation using *Saccharomyces* yeast extracted from toddy. *Journal of Chinese Institute of Chemical Engineers*, 34, 487-492.

50- Kongkiattikajorn, J. (2012). Ethanol production from dilute-acid pretreated cassava peel by fed-batch simultaneous saccharification and fermentation. *International Journal of the Computer, the Internet and Management*, 20(2), 22-27.

51- Walker, G.M. (2010). Bioethanol: science and technology of fuel alcohol. GM Walker and Ventus Publishing ApS. 114p.