

## Original Research Article

### BIOHYDROLYSIS OF BANANA AND PLANTAIN PEELS FOR THE PRODUCTION OF BIOFUEL

#### ABSTRACT

This study was carried out to assess the potentials of banana and plantain peel as **feedstock** **feedstocks** for biofuel production. Fungi were isolated from spoiled banana, burkutu and spoiled bread using the standard microbiological method. The concentration of reducing sugar of the peels were measured using DNS calorimetry method and biofuel were measured using chromium (VI) reagent by Ultraviolet-Visible Spectrophotometer. *Aspergillus niger*, *Saccharomyces cerevisiae* and *Mucor racemosus* were isolated. A reducing sugar concentration of 59.12 mg/g and 56.62 mg/g was observed for the banana and plantain peels. The highest concentration was found to be 0.35 mg/L for banana peels and 0.10 mg/L for plantain. The IR characterization of the banana and plantain sample revealed an intense strong broad band of alcohol O-H and alkane C-H stretching. The GC-MS result revealed the presence of benzaldehyde in all the biomass while 2,3-butanediol was only detected in the plantain peels biomass. This study showed the potential of banana and plantain peels biomass for biofuel production.

**Keyword:** Biofuel, banana peels, plantain peels, hydrolysis, *Saccharomyces cerevisiae*, *mucor racemosus*.

#### INTRODUCTION

Biofuel has been used in large scale since the implementation of Brazilian alcohol program (Goldemberg, 2008). The production of biofuel from lignocellulosic materials ~~are~~ also called Second-generation, is proposed as an alternative without ~~such~~-adverse effects ~~and~~. These fuel include Bioethanol, Biodiesel, Bioether, Biogas etc (Pimentel *et al.*, 2005). In Nigeria, all stages of the fruit (from immature to over ripe) are used as a source of food in one form or the other. Banana and plantain peels represent 40% of the total weight of fresh banana or plantain. Musa species are rich in vitamin C, B<sub>6</sub>, minerals and dietary fiber. They are also a rich energy source, with carbohydrates accounting for 22 % and 32 % of fruit weight for banana and plantain (Tchobanoglous *et al.*, 1993). Biofuels are considered as a replacement for fossil fuels and the answer to poverty and even the climate crisis. They are presented as being both renewable and environment friendly (Bassey, 2010). Increasing attention is being focused on the production of biofuels as the alternatives that will contribute to global reduction in greenhouse gas emissions (Oniemola and Sanusi, 2009). **Currently**, there is a growing interest for ecological sustainable bio-fuels all over the world. The aim of this research is to consider the possibility of

37 biohydrolysis of banana and plantain peels for biofuel production. ~~Which~~ which are common  
38 agricultural wastes in this part of the world. ~~and the~~ The objectives of this study were herein is  
39 to isolate and identify *Saccharomyces cerevisiae* from burukutu, *Aspergillus niger* and *Mucor*  
40 *racemosus* from spoiled banana and bread respectively. Biological pretreatment ~~carryout~~ with  
41 *Mucor racemosus* and reducing sugar contents were determine from banana and plantain peels  
42 sample and finally to identify the biofuel produced in banana and plantain feedstock  
43 fermentation broth using Gas Chromatography, ~~and~~ Mass spectroscopy(GC-MS) and Fourier  
44 Transform Infra red (FT-IR).

## 45 **MATERIALS AND METHODS**

### 46 **Sample Collection**

47 Banana (*Musa acuminata* - colla) and plantain (*Musa paradisiaca* - linnaeus) peels were  
48 collected from fruit vendors at Gawon-nama area, Sokoto metropolis. The banana and plantain  
49 peels were washed with distilled water to remove dirt and other contaminants. The samples were  
50 chopped into small pieces and allowed to air dried. It was grounded to powdered form using  
51 mortar and pestle and collected in a transparent polythene bag .

### 52 **Isolation and identification of *Aspergillus niger***

53 Small portion of spoiled banana was aseptically picked with wire loop and inoculated at the  
54 middle of the prepared media PDA (Potato dextrose agar ) plate. The plate were incubated at  
55 27°C temperature for 7 days for colonies development. Pure culture of *Aspergillus niger* was  
56 obtained by streak plate method. All observed colonies were maintained on agar slants at 4°C  
57 (Devanathan *et al.*, 2007) .

### 58 **Isolation and identification of *Sacchararomyces cerevisiae***

59 The burukutu samples were serially diluted up to 10<sup>5</sup> tubes. An Aliquot of 0.1 mL of burukutu  
60 was spread on the surface of yeast potatoes dextrose agar (YPDA) plate and incubated at 30°C  
61 for 3 days. Colonies suspected to be *Sacchararomyces cerevisiae* based on colonial  
62 characteristics were sub-cultured on PDA. A smear of the isolate was examined microscopically  
63 after staining. The isolates were identified by comparing their characteristics with those of  
64 known taxa using the schemes of Domsch and Grams (1970), as described by Oyeleke and  
65 Manga (2008) .

66 **Isolation and identification of *Mucor racemosus***

67 A small portion of the spoiled bread was aseptically picked with wire loop and inoculated at the  
68 middle of prepared solidified media sabourand dextrose agar (SDA). It was incubated at 30 °C  
69 for 5 days. Colonies suspected to be *Mucor racemosus* based on colonial characteristics were sub  
70 cultured on PDA. For identification, One (1) drop of lectophenol blue was dropped on dried  
71 grease free slide, little portion of the growth was picked with inoculated needle and emulsified.  
72 Cover slide was used to cover and viewed at 10x objective and confirmed by 40x (David *et*  
73 *al.*,2007).

74 **Biological pretreatment**

75 The biological pretreatment was carried out as described by Ekunsaumi (2006). Mandels culture  
76 medium was prepared by adding (gl<sup>-1</sup>): Urea 0.3,(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.4, KH<sub>2</sub>PO<sub>4</sub> 2,CaCL<sub>2</sub> 0.3,  
77 MgSO<sub>4</sub>·7H<sub>2</sub>O 0.3, bacto peptone 0.75, and yeast extract 0.25. Trace elements was also added,  
78 using a 1% (v/v) solution of salts (ml-1):FeSO<sub>4</sub>·7H<sub>2</sub>O 0.5, MNSO<sub>4</sub> 0.16, ZnSO<sub>4</sub> 0.14, CoCl<sub>2</sub> 2g at  
79 pH of 5.5. The medium was measured as 100 mL, 150 mL and 200 mL and the substrate (banana  
80 and plantain peels) were added as 5g, 10g and 15g into 250 mL Erlenmeyer conical flasks. The  
81 medium was then sterilized by autoclaving at 121 °C at 15 minutes. All the flasks was inoculated  
82 with 1 mL of the test organism (*Mucor racemosus*). The flasks were incubated at 28°C ambient  
83 laboratory temperature on an orbital shaker at 200 rpm for 5 days. After 5 days the mixture was  
84 separated by filtration through a Whatman filter paper no 1. The filtrate was then used for further  
85 studies.

86  
87 **Enzymatic Hydrolysis**

88 Enzymatic hydrolysis was carried out on the biological pretreated samples. Distilled water was  
89 dispensed into the substrate across the labeled flask and heated gently on a hot plate to ensure  
90 dissolution. The mixture was autoclaved at 121°C for 15 minutes for sterilization. After cooling  
91 at room temperature of 30°C it was inoculated with *Aspergillus niger* suspension using  
92 Mcfarland turbidity standard at 0.5, 1.0 and 1.5 mL using sterile syringe. The flasks were  
93 incubated at 28°C for five (5) days for hydrolysis to take place. Then, Concentration of reducing  
94 sugar was quantified from the hydrolysate obtained from enzymatic using dinitrosalicylic  
95 colorimetric method (DNS) as described by (Oyeleke *et al.*, 2012).

96 **Determination of Reducing Sugar**

97 After hydrolysis the sample were filtered using Whatman's filter paper no 1 and presence of  
98 reducing sugar in each sample was determine using dinitrosalicylic colorimetric DNS (Miller,  
99 1959). One (1.0) mL of the sample was added to 1 mL of 3,5 dinitrosalicylic acid, followed by  
100 boiling for 10 minutes. The absorbance was measured at 540 nm with a spectrophotometer  
101 (Jenway 6100).

102 **Fermentation of Hydrolysate**

103 To obtain a broth after the hydrolysis step, the fermentation media was filtered using whatman  
104 no 1 filter paper. The flasks containing the filtrate was covered with cotton wool wrapped in  
105 aluminum foil then autoclaved at 121°C for 15 minutes. The sterile hydrolysate was allowed to  
106 cool and *Saccharomyces cerevisiae* suspension were inoculated at 0.5, 1.0 & 1.5 mL and  
107 incubated at 30°C ambient temperature for 5 days to monitor ethanol production.

108 **Distillation**

109 The fermented broth were filtered using Whatman no 1 filter paper. Each sample were weighted  
110 into kjeldahl flasks and heated at 78°C (boiling point of ethanol) on the Microkjeldahl apparatus  
111 until solution turns colorless.

112 **Determination of biofuel Quality**

113 Determination of biofuel quality was according to the method described by Patel *et al.*  
114 (2007). This was carried out using UV-VIS quantitative analysis of alcohol using chromium VI  
115 reagent. A quantity (1ml) of standard ethanol was diluted with 100 mL of distilled water to give  
116 a concentration of 1%. Then, each of 0, 2, 4, 6 and 8 mL of the ethanol was diluted to 10ml with  
117 distilled water to produced 0, 0.2, 0.4, 0.6 and 0.8% of the ethanol. To each of the varying  
118 ethanol concentrations 2 mL of chromium reagent was added and allowed to stand for an hour  
119 for colour development. The absorbance of each concentration were measured at 588 nm using  
120 UV -VIS spectrophotometer and the readings used to developed standard ethanol curve. Then  
121 1ml of each biofuel samples were put into test tubes and treated with 2 mL of the chromium  
122 reagent. The mixture was allowed to stand for an hour and the absorbance measured at 588 nm  
123 using the Ultraviolet-Visible spectrophotometer (Rabah *et al.*, 2011).

124 **Determination of compounds present in the Biofuel produced**

125 Gas Chromatography and mass spectrometry (GC-MS) analysis of the produced bio-ethanol was  
126 conducted at General Science Laboratory, Usmanu Danfodiyo University, Sokoto on Agilent  
127 technologies 6890N Network GC System and Agilent technologies 5973 network mass selective  
128 detector coupled with 7683B series injector. During the analysis, the oven temperature was  
129 maintained at 50°C for 5 minutes. The injector and detector temperature were 120°C and 160°C  
130 respectively. The flow rate for carrier gas (Helium) was set at 1.2ml/min and the injection  
131 volume were 1NL (Mei-ling *et al.*, 2003). The inlet temperature was maintained at 230 °C. The  
132 oven was programmed again to increase to 300°C at a rate of 10°C ending with 25 minutes and  
133 this temperature ~~is to be held~~ was held for 15 minutes, total runtime were 45 minutes. The mass  
134 spectrometry (MS) transfer line were maintained at a temperature of 250<sup>0</sup> C. The source  
135 temperature were also maintained at 230°C and Ms quad at 150°C. The ionisation mode to be  
136 used is electron ionization mode at 70 Ev. Total Ion count (TIC) was used to evaluate for  
137 compound identification and quantification. The spectrum of the separated compound were  
138 compared with the database of the spectrum of known compound saved in the compared with the  
139 database of the spectrum of known compound saved in the NISTO2 Reference Spectra Library.  
140 Agilent chemstation software was used to carry out data analysis and peak area.

#### 141 **Fourier Transform Infrared Analysis of the produced Biofuel**

142 The pellet technique as described by Gershon, *et al.*, (1981) was used. FT-IR spectra were taken  
143 on a Kb 3000 series (Shimadzu, Kyoto, Japan) spectrophotometer using the KBr pressed disk  
144 technique, ethanol was used to clean the KBr cells. The machine calibration was tested by  
145 applying drops of nujol (liquid paraffin) solvent to a KBr cell and run its Infrared to ascertain its  
146 conformity with existing spectra. A few drops of the sample was added and 3 drops of nujol  
147 solvent were used to dissolve it in a mortar. The paste formed was pressed on the KBr cell and  
148 inserted into the machine for 10 Mpa for 3 minutes, the spectra were recorded over the spectral  
149 range between 4000 and 400 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>.

#### 150 **Statistical Analysis**

151 All the work experiments were conducted in triplicates. All data obtained is expressed as mean  
152 and standard deviation.

#### 153 **RESULTS**

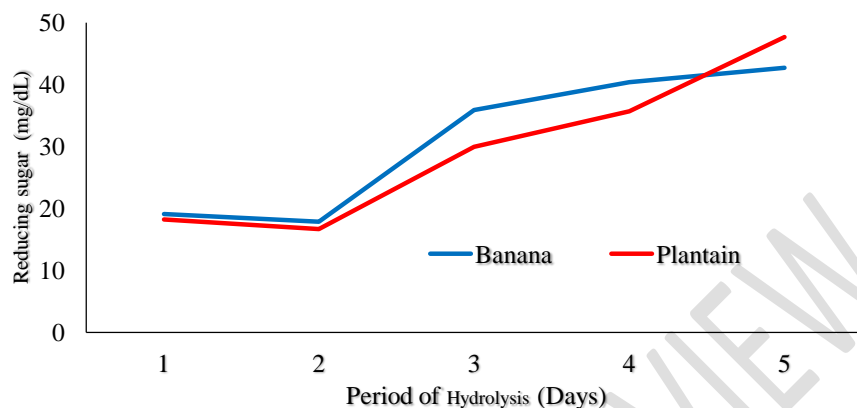
154 The results presented in Table 1 shows the phenotypic characteristics of *Aspergillus niger*  
 155 isolated from spoiled banana and *Saccharomyces cerevisiae* isolated from 'burukutu' as well as  
 156 *Mucor racemoses* isolated from spoiled bread. *Aspergillus niger* showed a black mycelium on  
 157 the agar medium and presence of conidial head. *Saccharomyces cerevisiae* was identified as  
 158 having creamish colonies with white buds forming cluster of cell.

159 Table 1: Phenotypic identification of fungi isolated from Burukutu, spoilt banana and bread

Sample	Macroscopic	Microscopic	Organism
Spoiled banana	White colonies with dense layer of dark brown Presence of black conidiophores	Presence of conidial heads Conidiophore stripe smooth walled Hyaline brown color	<i>Aspergillus niger</i>
Burukutu	White dense layer	White buds, forming cluster of cell.	<i>Saccharomyces cerevisiae</i>
Spoiled Bread	Colony white presences of tall and short Sporangiophores Sporangia hyaline becoming brownish to gray with age	Columella obovoid ellipsoidal Sporangiospore broadly ellipsoidal Smooth-walled grayish	<i>Mucor racemosus</i>

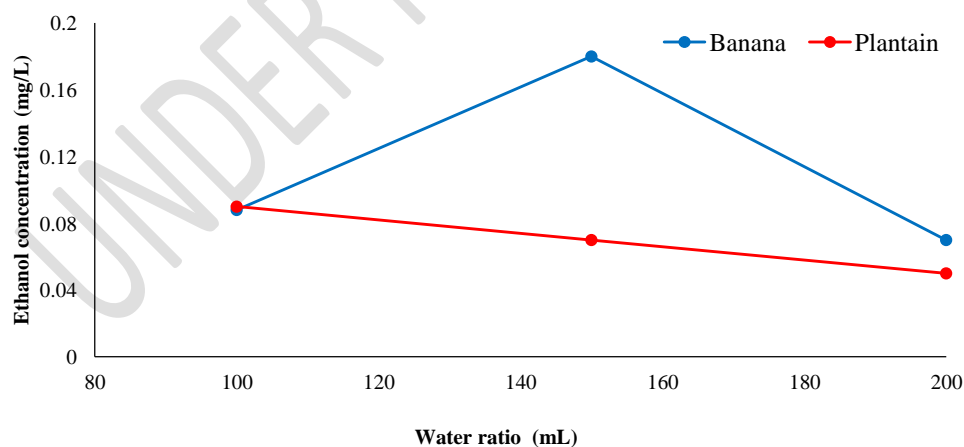
160  
 161 The results of effect of hydrolysis on reducing sugar content from banana and plantain peels as  
 162 substrate are presented in Figure 1. It was observed that at 5<sup>th</sup> day of hydrolysis the highest  
 163 yields of reducing sugar of 59.12 and 56.62 mg/dL were obtained from banana and plantain  
 164 peels respectively with water ratio of 200 mL, 15g of substrate ratio and 1.5 mL of inoculum  
 165 size using *Aspergillus niger*. And 2<sup>nd</sup> day of hydrolysis, lowest concentration yield of reducing  
 166 sugar (16.10 mg/dL) was obtained from plantain peels with water level of 150 mL, 10 g of  
 167 substrate and 1.0 mL of inoculum of *Aspergillus niger*. While 1<sup>st</sup> day showed the lowest  
 168 concentration yield of reducing sugar of 16.35 mg/dL obtained from banana peels with water  
 169 level of 100 mL, 5 g of substrate and 0.5 mL of inoculums.

170  
 171



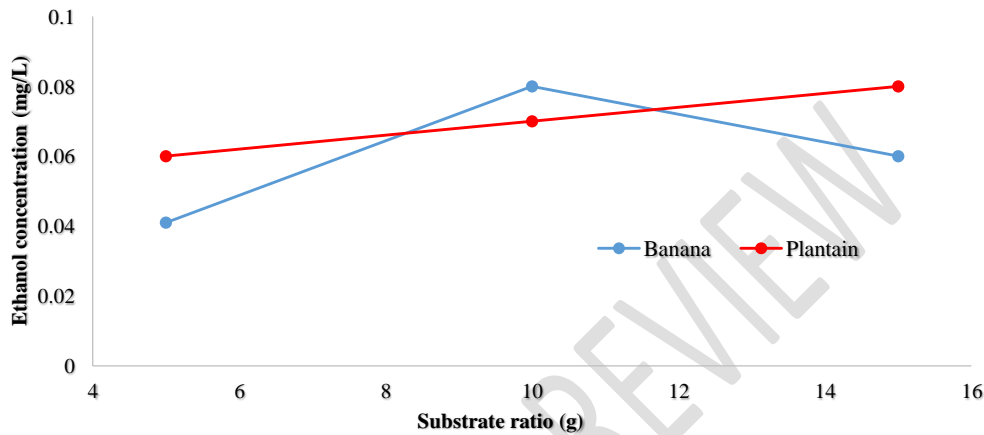
172 **Figure 1: Effect of Hydrolysis on reducing sugar content from banana and plantain peels**  
 173 **as substrate.**  
 174

175 The effect of water ratio on biofuel production from banana and plantain peels using  
 176 *Saccharomyces cerevisiae* indicated a decreased in biofuel concentration with increase water  
 177 ratio figure 2. It was observed that the plantain produced highest ethanol at 100 mL water ratio  
 178 and gradually declined toward 200 mL, while the banana peel produced highest ethanol at 150  
 179 mL.



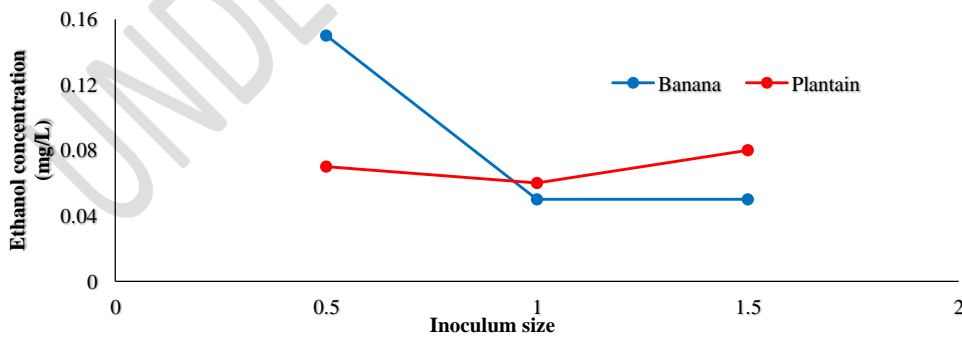
180 **Figure 2: Effect of water ratio on biofuel yield using banana and plantain peels as**  
 181 **substrate at the 5<sup>th</sup> days of fermentation**  
 182

183 The result of effect of banana and plantain peels concentration on biofuel yield is presented in  
 184 Figure 3. It was observed that substrate concentration increased from 5 to 15 g/L and biofuel  
 185 yield increased to 0.08 mg/L.



186 **Figure 3: Effect of substrate ratio on biofuel yield using Banana and plantain peels as a**  
 187 **substrate at the 5<sup>th</sup> days of fermentation.**  
 188  
 189

190 The effect of inoculum size on biofuel production of banana and plantain peels using  
 191 *Saccharomyces cerevisiae* is presented in Figure 4. It was observed that 0.5 mL inoculum size of  
 192 *Saccharomyces cerevisiae* produced highest yield of 0.16 mg/mL ethanol on banana peels. While  
 193 on plantain peels *Saccharomyces cerevisiae* produced moderate yield of 0.09 mg/mL at 1.5 mL  
 194 of inoculum size.



195  
 196 **Figure 4: Effect of inoculum size on biofuel yield using banana and plantain peels as**  
 197 **substrate at the 5<sup>th</sup> days of fermentation**

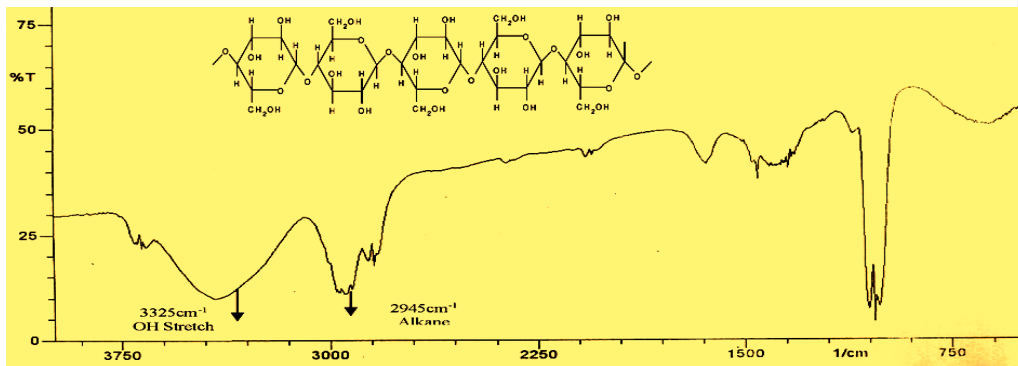


198 The volatile organic metabolites of banana and plantain peels were hydrolysed using *A. niger*  
 199 and fermented for biofuel production. Banana peels had high abundance of benzaldehyde  
 200 (22.20%), which was present in the plantain peel, and the plantain peels had the biofuel '2,3-  
 201 butanediol' while 9-Octadecenoic, Methyl ester (18.80%) had the highest abundance. The results  
 202 is presented in Table 3.

203  
 204 **Table 3: Volatile organic metabolite of banana and plantain peels fermented for biofuel**  
 205 **production potential**

Retention Time(min.)	Organic metabolite profile	Abundance (%)	
		Banana	Plantain
2.315	2,3-Butanediol,	-	1.66
3.019	Benzaldehyde	22.20	8.63
6.705	Caprolactam	-	1.18
14.047	4-Benzylaniline	3.85	4.30
15.431	Hexadecanoic acid, methyl ester	4.84	5.36
16.856	9-Octadecenoic acid, methyl ester, (E)-	14.52	18.80
17.057	Methyl stearate	13.95	16.42
17.186	Oleic Acid	4.66	4.54
17.366	Octadecanoic acid	7.58	8.58
18.520	Eicosanoic acid, methyl ester	1.00	0.76
19.190	9-Octadecenoic acid (Z)-	0.43	1.27
19.358	Octadecanoic acid, 2,3-dihydroxypropyl ester	0.34	2.39
19.512	Z,Z-4,16-Octadecadien-1-ol acetate	2.08	2.39
19.675	Glycidol stearate	2.30	2.46
19.741	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2.32	1.40
20.136	Butyl 14-methylhexadecanoate	1.24	7.72
20.892	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	8.36	12.14
21.038	Octadecanoic acid, 2,3-dihydroxypropyl ester	10.34	4.54

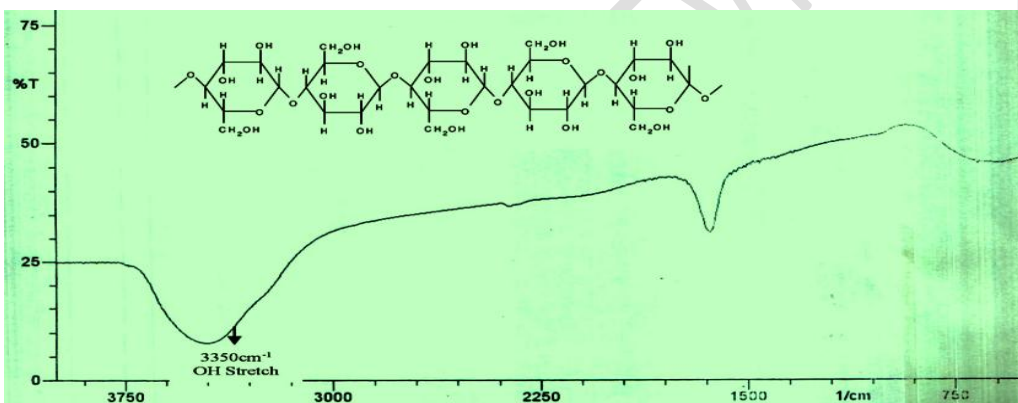
206  
 207  
 208 The result of Infrared spectroscopy for characterization of banana and plantain peels from the  
 209 produced biofuel at (figure 5 and 6 ) shows an O-H characteristics stretch with an intense and  
 210 broad band of  $3500\text{cm}^{-1}$  to  $2200\text{cm}^{-1}$  and  $1260\text{cm}^{-1}$  to  $1050\text{cm}^{-1}$  respectively corresponding to  
 211 alcohol.



212  
213  
214

Figure 5 : FT-IR spectrum of the distillate obtained from fermented banana peels.

215



216  
217  
218

Figure 6: FT-IR spectrum of the distillate obtained from fermented plantain peels.

219

## 220 DISCUSSION

221 The results from this study indicated *Aspergillus niger*, *Mucor racemosus* and *Sacchromyces*  
 222 *cerevisiae* were isolated from spoiled banana, bread and burukutu. It is not surprising the  
 223 organisms are found on the samples as genus *Aspergillus* are filamentous fungi which are  
 224 ubiquitous and of great importance in medical and industrial microbiology. They comprise of  
 225 species and strains such as *Aspergillus niger* that secrete copious amounts of cellulolytic  
 226 enzymes (Acharya *et al.*, 2008). Essien *et al.*, (2005) reported banana fruit peel could support  
 227 microbial growth thus it could be used as a substrate for the production of variable micro fungal

228 biomass. The result is in conformity with Yahaya and Ado (2008) who reported the mycelial  
229 protein production of *Aspergillus niger* using banana peels. *Saccharomyces cerevisiae* was  
230 isolated from "burukutu" in this studies. *Saccharomyces cerevisiae* is the organism of choice as  
231 the fermentation process biocatalyst in alcoholic beverages and fuel ethanol industry (Vallet *et*  
232 *al.*, 1996). The organism was able to ferment glucose, fructose, Sucrose, maltose and galactose  
233 producing acid and gas. This results agree with Elijah *et al.* (2010) who reported the isolation  
234 *Saccharomyces cerevisiae* among other yeast from palm wine.

235 ~~In this work biological pretreatment of banana and plantain peels was performed using *Mucor*  
236 and enzymatic hydrolysis with *Aspergillus niger* to breakdown the lignocellulosic component of  
237 our biomass to expose the simple sugar for further fermentation to produce alcohol. Reducing  
238 sugar is utilize by yeast for biofuel production (Das and Singh, 2004).~~

**Comment [R.U1]:** This is not discussion of results

239 At day 5 the highest yield of reducing sugar of 59.12 and 56.62 mg/dL was obtained from  
240 banana and plantain respectively with water ratio of 200 mL, 15g of substrate ratio and 1.5 mL  
241 of inoculums size using *Aspergillus niger*. This result might be due to high lignocellulosic  
242 biomass contained in the substrate that can be converted to reducing sugar which later  
243 metabolize by yeast to ethanol, but it cannot produce cell enzymes which is required to degrade  
244 the cellulose, present in the banana peels (Essien *et al.*, 2005). *Aspergillus* spp are able to  
245 produce different kind of enzymes e.g amylase, cellulase and protease which is responsible for  
246 degradation of polysaccharide into monosaccharide (Auta *et al.*, 2012).

247 At day 2 lowest concentration yield of reducing sugar (16.10 mg/dL) was obtained from plantain  
248 peels with water level of 150 mL, 10g of substrate and 1.0 mL of inoculum using *Aspergillus*  
249 *niger* and day 1 showed the lowest concentration yield of reducing sugar of 16.35 mg/dL was  
250 obtained from banana peels with water level of 100 mL, 5g of substrate and 0.5 mL of  
251 inoculums. It is not surprising because at the lag phase cell synthesis enzymes that could convert  
252 readily available sugar, whereby the organisms undergo cell repair by adapting to the  
253 environment. This is in agreement with the results of Laopaiboon *et al.* (2010), they reported  
254 that the hydrolysis of sugarcane baggasse (SCB) for lactic acid production using HCl is quite  
255 slow compared to when using H<sub>2</sub>SO<sub>4</sub>, maximum total sugar and reducing sugar concentration of  
256 49.95 and 21.33 g/c were obtained when using 5% (v/v) of H<sub>2</sub>SO<sub>4</sub>. While in contrary to Itelima

257 *et al.* (2013) the highest reducing concentration (0.94mg/cm<sup>3</sup>). *Trichoderma reesei* released  
258 maximum reducing sugar of 22.30 mg/g in paddy straw, 25.56 mg/g in wheat straw, and 28.26  
259 mg/g in sugarcane bagasse. *Phanerochaeta chrysosporium* recorded reducing sugars of 14.55  
260 mg/g in paddy straw, 18.11 mg/g in wheat straw and 20.96 mg/g.

261 The effect of water ratio on biofuel production from banana and plantain peels sample using  
262 *Saccharomyces cerevisiae* indicated a decrease in bio ethanol concentration with increase water  
263 ratio. The decrease in ethanol concentration with increase in water ratio observed for plantain is  
264 likely due to water activity. As previously stated that water activity is the key variable to ethanol  
265 concentration for the description of some of the nonspecific inhibitory effects apparent in ethanol  
266 fermentation (Jones and Greenfield, 1986). The peak ethanol concentration observed at water  
267 ratio of 1:15 suggests that it is the peak at which the ethanol exact the lowest water activity.  
268 Ethanol induced water activity stress has been previously reported to exact stress on yeast  
269 metabolism and cell wall integrity (Hallsworth, 1998). These findings are in conformity with the  
270 work of Epstein *et al.* (2010) who reported an ethanol volume as low as 0.06 g/mL from apple  
271 and grape juices.

272 The effect of banana and plantain peels concentration on biofuel yield indicated substrate  
273 concentration increase from 5 to 15 g/L, ethanol yield increased to 0.08 mg/L. The decrease in  
274 ethanol yield beyond the optimum concentration 0.05 mg/L could be as a result of product  
275 inhibition. This implies that the ethanol produced inhibits the activity of the yeast, at low  
276 substrate levels, an increase of substrate concentration normally results in an increase of the yield  
277 and reaction rate of the hydrolysis (Cheung and Anderson, 1997). However, high substrate  
278 concentration can cause substrate inhibition, which substantially lowers the rate of the  
279 hydrolysis, and the extent of substrate inhibition depends on the ratio of total substrate to total  
280 enzyme (Huang and Penner, 1991; Penner and Liaw, 1994). These findings are in conformity  
281 with the work Reddy *et al.* (2010) who carried out an investigation on use of banana agro waste  
282 for the ethanol production. They used cellulolytic thermophilic *Clostridium thermocellum* CT2  
283 for the biological production of ethanol. This culture was isolated from elephant droppings. They  
284 obtained the maximum ethanol yield of 0.41 g/g substrate used.

285 The increase in fuel yield may be due to continuous excretion of enzymes by the microorganism  
286 in the solution. The decrease in fuel yield beyond the optimum concentration could be that at the  
287 high substrate concentration, the active sites of the enzyme molecules at any given moment were  
288 virtually saturated with substrate. Thus any extra substrate has to wait until the enzyme complex  
289 has released the products before it may itself enter the active site of the enzyme (Wen, 2004).

290 The effect of inoculum size on biofuel production of banana and plantain peels sample using  
291 *Saccharomyces cerevisiae* indicated an increase in ethanol production. A study done by  
292 Mahamed and Reddy (1986) reported that the increasing *Saccharomyces cerevisiae* inoculums in  
293 the co-cultures *Aspergillus niger* and *Saccharomyces cerevisiae* from 4% to 12% showed an  
294 increase in the rate ethanol production from potato starch. Ocloo and Aywnor (2010) also  
295 reported that the time taken for the fermentation to be completed was affected significantly by  
296 the yeast concentration. The result obtained supported the fact that the speed of fermentation  
297 depends on the yeast concentration and the shorter the fermentation period required to achieve  
298 maximum alcohol yield (kordylas, 1990). Ueda *et al.*, (1981) reported 5days fermentation for  
299 raw cassava root starch using 15% yeast suspension. Togarepi *et al.*, (2012) reported increase  
300 production rate rapidly with the increase in the amount of yeast to the concentration of 8g/20g  
301 fruit pulp. Beyond that point the rates no longer significantly increased. At this point the  
302 substrate becomes limiting and increase the yeast amount does not increase the rate of reaction.

303 The FT-IR on the produced alcohol are shown on Fig. 5 have an alcohol characteristic IR  
304 absorption associated with O-H and C-H stretching vibrations. When run as a liquid film the  
305 region 3550-3200 $\text{cm}^{-1}$  region correspond to O-H stretching and C-H stretching region 2850-  
306 3000 $\text{cm}^{-1}$ . The spectra of the banana peels shows the band at around 3325  $\text{cm}^{-1}$  and 2945  $\text{cm}^{-1}$   
307 for phenol/alcohol O-H stretch and alkane C-H stretching respectively with a very intense and  
308 strong broad band. The broad O-H correspond to peak for O-H from alcohol. This might be the  
309 alcohol group of 2,3-butanediol. The C-H stretch that correspond to alkane might represent be  
310 the body compound such as Benzaldehyde. This is likely as a result of the various vibration  
311 modes that are found in carbohydrate and Lignin (Bodirlau *et al.*, 2008).  
312 The spectra of the plantain peels indicated the presence (OH) alcohol stretching at 3350  $\text{cm}^{-1}$  and  
313 intense strong broad. Although all samples were similar slight changes were observed possibly  
314 indicating that equivalent strategies should be employed for releasing fermentable sugar from

315 these lignocellulosics. And also the broad O-H peak was due to the interaction ( hydrogen  
316 bonding) and the water content. The samples consist of alkene, alcohol and alkenyl indicating the  
317 presence of hydrocarbons in the biofuel.

318 The biofuel 2,3-butanediol (2,3-BD) was detected in the distillate of the plantain biomass. 2,3-  
319 butanediol is a potential valuable fuel additive that has 27.2KJ/g heat value. The heat value of  
320 2,3-BD is comparable to those of other liquid fuel such as ethanol which has 29.055KJ/g and  
321 methanol which has 22.081KJ/g (Flickinger,1980).The presences of 2,3-BD is not surprising as  
322 the yeast *S. cerevisiae* used for fermentation in this study has been previously shown to posses  
323 three different 2,3-BD biosynthetic pathway (Ng *et al.*, 2012).

#### 324 **Conclusion**

325 This study concludes that banana and plantain peels has potential as a sustainable and low-cost  
326 biomass for the production of biofuel such as 2,3-butanediol (2,3-BD). *Aspergillus niger*,  
327 *Sacchromyces cerevisiae* and *Mucor racemosus* were Identified. A reducing sugar  
328 concentration was observed for the banana and plantain peels. The biofuel concentration was  
329 found to be highest in banana and plantain peels. The IR characterization of the sample revealed  
330 with intense strong broad band of alcohol O-H and alkane C-H stretching respectively. GC-MS  
331 result revealed the presence of benzaldehyde in all the biomass while 2,3-butanediol was only  
332 detected in the plantain peels biomass. Burukutu can be exploited for sufficient *Saccharomyces*  
333 *cerevisiae* for biofuel production. This study shows the potential of bioconversion of banana and  
334 plantain peels biomass for biofuel production.

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