**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 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 racemusus* 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 racemus.*


## 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, $B_6$, 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 biohydrolysis of

UNDER PEER REVIEW

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

44 **MATERIALS AND METHODS**

45 **Sample Collection**

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

51 **Isolation and identification of *Aspergillus niger***

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

57 **Isolation and identification of *Sacchararomyces cerevisiae***

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

65 **Isolation and identification of *Mucor racemosus***



UNDER PEER REVIEW

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

73 **Biological pretreatment**

74 The biological pretreatment was carried out as described by Ekunsaumi (2006). Mandels culture
75 medium was prepared by adding ($gl^{-1}$): Urea 0.3,$(NH_4)2SO_4$ 1.4, $KH_2PO_4$ 2,$CaCL_2$ 0.3,
76 $MgSO_47H_2O$ 0.3, bacto peptone 0.75, and yeast extract 0.25. Trace elements was also added,
77 using a 1% (v/v) solution of salts (mll-1):$FeSO_47H_2O$ 0.5, $MNSO_4$ 0.16, $ZnSO_4$ 0.14, $CoCl_2$ 2g at
78 pH of 5.5. The medium was measured as 100 mL, 150 mL and 200 mL and the substrate (banana
79 and plantain peels) were added as 5g, 10g and 15g into 250 mL Erlenmeyer conical flasks. The
80 medium was then sterilized by autoclaving at 121 $^{O}$C at 15 minutes. All the flasks was inoculated
81 with 1 mL of the test organism (*Mucor racemuses*). The flasks were incubated at 28°C ambient
82 laboratory temperature on an orbital shaker at 200 rpm for 5 days. After 5 days the mixture was
83 separated by filtration through a Whatman filter paper no 1. The filtrate was then used for further
84 studies.
85

86 **Enzymatic Hydrolysis**

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

95 **Determination of Reducing Sugar**



UNDER PEER REVIEW

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

101 **Fermentation of Hydrolysate**

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

107 **Distillation**

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

111 **Determination of biofuel Quality**

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

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

UNDER PEER REVIEW

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

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

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

**Statistical Analysis**

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

**RESULTS**

UNDER PEER REVIEW

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

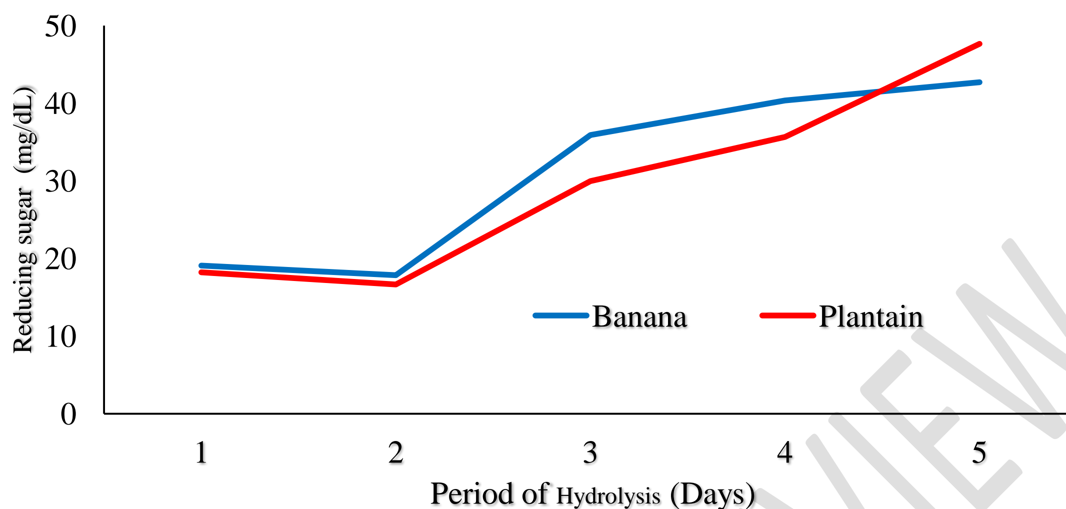
158 Table 1: Phenotypic identification of fungi isolated from Burukutu, spoilt banana and bread

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

159
160 The results of effect of hydrolysis on reducing sugar content from banana and plantain peels as
161 substrate are presented in Figure 1. It was observed that at 5$^{th}$ day of hydrolysis the highest
162 yields of reducing sugar of 59.12 and 56.62 mg/dL were obtained from banana and plantain
163 peels respectively with water ratio of 200 mL, 15g of substrate ratio and 1.5 mL of inoculum
164 size using *Aspergillus niger*. And 2$^{nd}$ day of hydrolysis, lowest concentration yield of reducing
165 sugar (16.10 mg/dL) was obtained from plantain peels with water level of 150 mL, 10 g of
166 substrate and 1.0 mL of inoculum of *Aspergillus niger*. While 1$^{st}$ day showed the lowest
167 concentration yield of reducing sugar of 16.35 mg/dL obtained from banana peels with water
168 level of 100 mL, 5 g of substrate and 0.5 mL of inoculums.

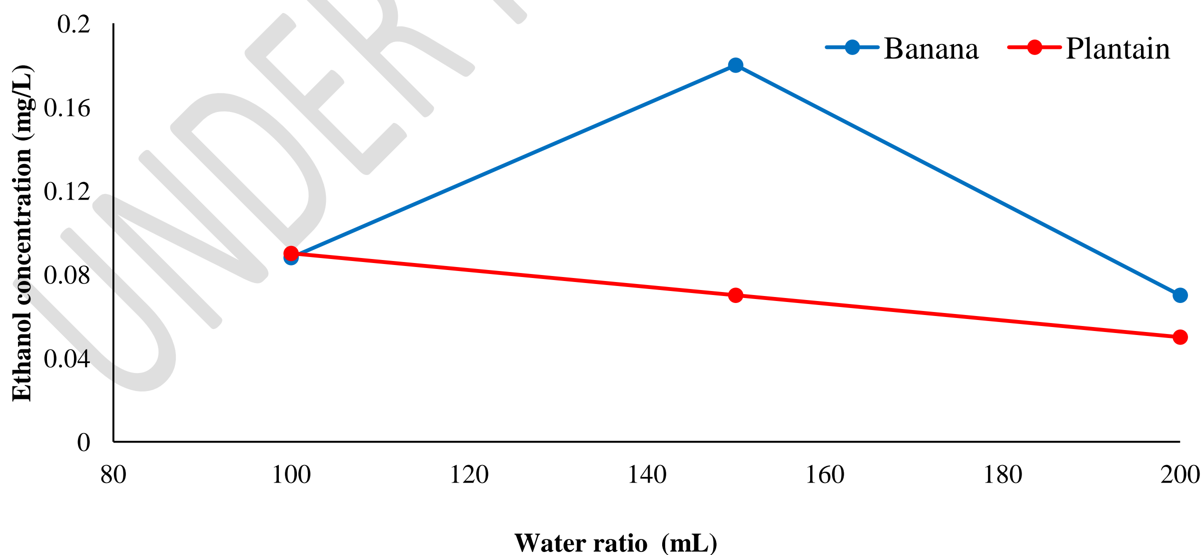
169
170

UNDER PEER REVIEW




171
172 **Figure 1: Effect of Hydrolysis on reducing sugar content from banana and plantain peels**
173 **as substrate.**

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

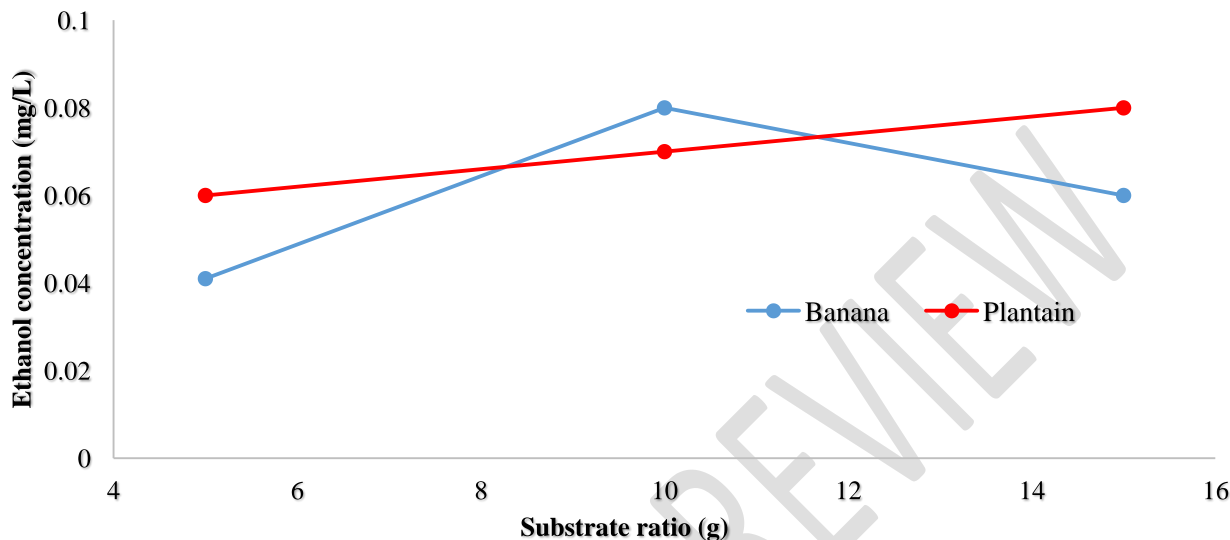



179
180 **Figure 2: Effect of water ratio on biofuel yield using banana and plantain peels as**
181 **substrate at the 5$^{th}$ days of fermentation**



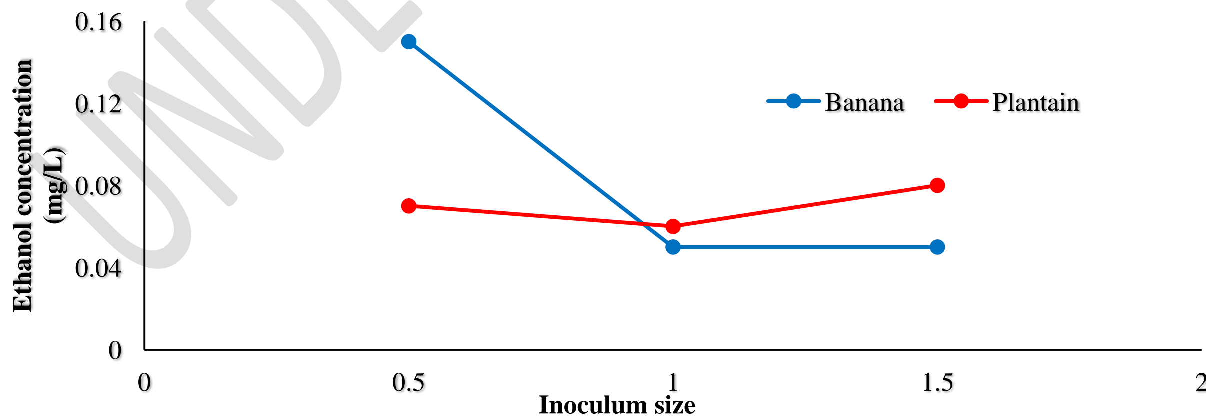
UNDER PEER REVIEW

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




185
186 **Figure 3: Effect of substrate ratio on biofuel yield using Banana and plantain peels as a**
187 **substrate at the 5th days of fermentation.**
188

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




194
195 **Figure 4: Effect of inoculum size on biofuel yield using banana and plantain peels as**
196 **substrate at the 5th days of fermentation**

UNDER PEER REVIEW

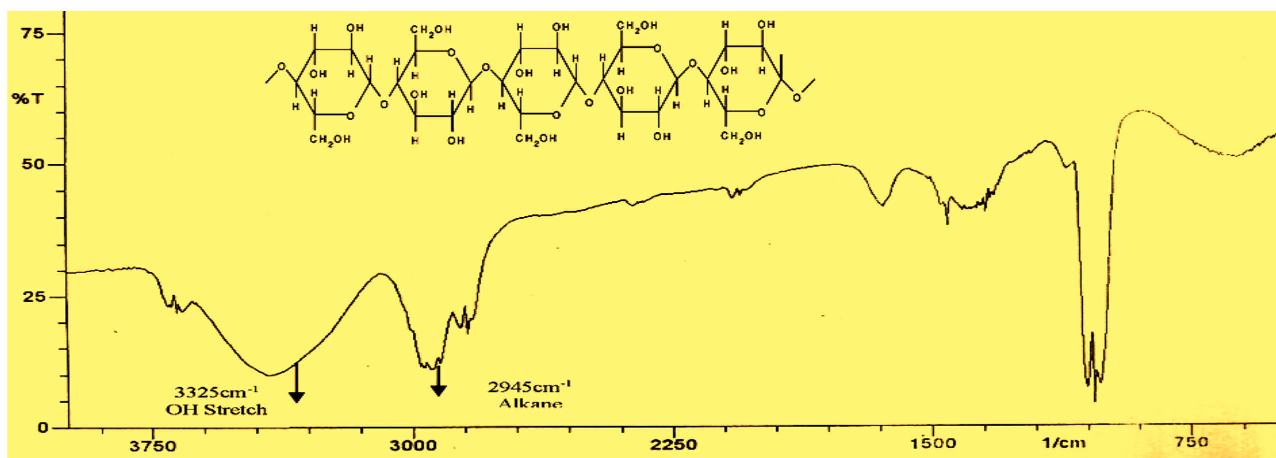
197 The volatile organic metabolites of banana and plantain peels were hydrolysed using *A. niger*
198 and fermented for biofuel production. Banana peels had high abundance of benzaldehyde
199 (22.20%), which was present in the plantain peel, and the plantain peels had the biofuel '2,3-
200 butanediol' while 9-Octadecenoic, Methyl ester (18.80%) had the highest abundance. The results
201 is presented in Table 3.
202
203 **Table 3: Volatile organic metabolite of banana and plantain peels fermented for biofuel**
204 **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 |

205
206
207 The result of Infrared spectroscopy for characterization of banana and plantain peels from the
208 produced biofuel at (figure 5 and 6 ) shows an O-H characteristics stretch with an intense and
209 broad band of $3500cm^{-1}$ to $2200cm^{-1}$ and $1260cm^{-1}$ to $1050cm^{-1}$ respectively corresponding to
210 alcohol.

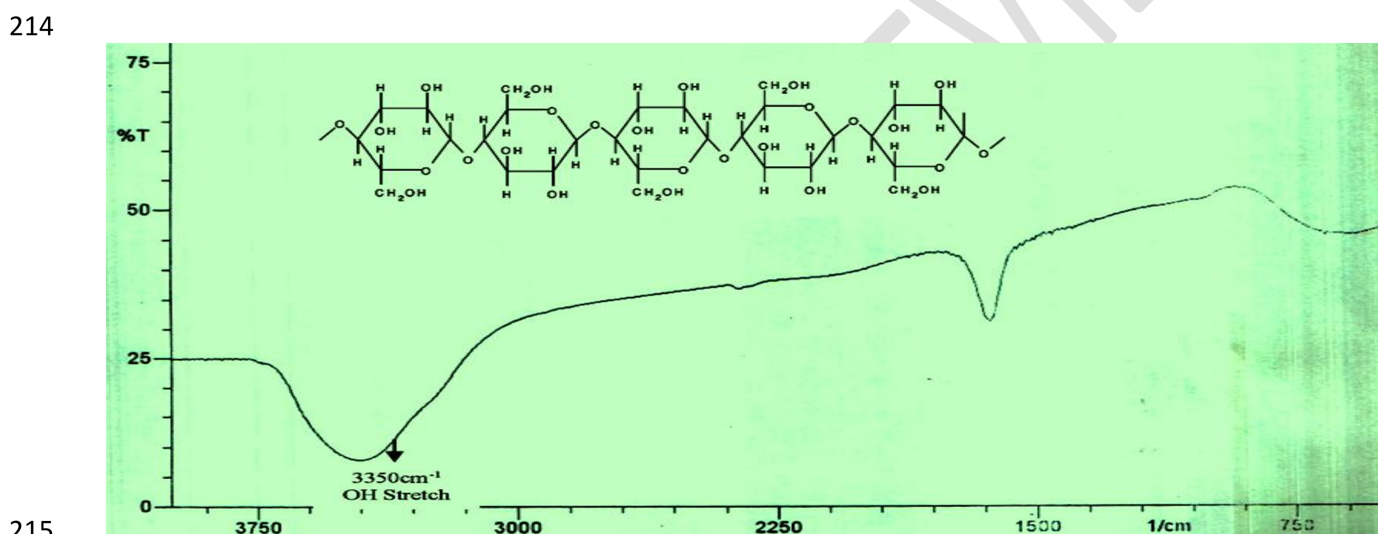
UNDER PEER REVIEW

211
212




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

214




215
216

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

218

219 **DISCUSSION**

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

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

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

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

246 At day 2 lowest concentration yield of reducing sugar (16.10 mg/dL) was obtained from plantain
247 peels with water level of 150 mL, 10g of substrate and 1.0 mL of inoculum using *Aspergillus*
248 *niger* and day 1 showed the lowest concentration yield of reducing sugar of 16.35 mg/dL was
249 obtained from banana peels with water level of 100 mL, 5g of substrate and 0.5 mL of
250 inoculums. It is not surprising because at the lag phase cell synthesis enzymes that could convert
251 readily available sugar, whereby the organisms undergo cell repair by adapting to the
252 environment. This is in agreement with the results of Laopaiboon *et al.* (2010), they reported
253 that the hydrolysis of sugarcane baggasse (SCB) for lactic acid production using HCl is quite
254 slow compared to when using $H_2S0_4$, maximum total sugar and reducing sugar concentration of
255 49.95 and 21.33 g/c were obtained when using 5% (v/v) of $H_2S0_4$. While in contrary to Itelima

UNDER PEER REVIEW

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

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

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

UNDER PEER REVIEW

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

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

302 The FT-IR on the produced alcohol are shown on Fig. 5 have an alcohol characteristic IR
303 absorption associated with 0-H and C-H stretching vibrations. When run as a liquid film the
304 region 3550-3200cm$^{-1}$ region correspond to 0-H stretching and C-H stretching region 2850-
305 3000cm$^{-1}$. The spectra of the banana peels shows the band at around 3325 cm-$^{1}$and 2945 cm$^{-1}$
306 for phenol/alcohol O-H stretch and alkane C-H stretching respectively with a very intense and
307 strong broad band. The broad O-H correspond to peak for O-H from alcohol. This might be the
308 alcohol group of 2,3-butanediol. The C-H stretch that correspond to alkane might represent be
309 the body compound such as Benzealdehyde. This is likely as a result of the various vibration
310 modes that are found in carbohydrate and Lignin (Bodirlau *et al.,* 2008).
311 The spectra of the plantain peels indicated the presence (OH) alcohol stretching at 3350 cm$^{-1}$ and
312 intense strong broad. Although all samples were similar slight changes were observed possibly
313 indicating that equivalent strategies should be employed for releasing fermentable sugar from

UNDER PEER REVIEW

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

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

323 **Conclusion**

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

334 **REFRENCES**

335 Acharya, P. B., Acharya, D. K. and Modi, H. A. (2008). Optimization for Cellulase Production
336 by *Aspergillus niger* using sawdust as substrate. *African Journal of Biotechnology,* **7** (22):
337 4147 – 4152.
338
339 Auta, H. S., Oyeleke, S. B., Ayodele, O. B.,Bala, J.D., Abioye O.P., Ibrahim, A. D., Ijah, U.J.J
340 and Manga, S. B.(2012).Characterization of extracellular amylase produced by *Aspergillus*
341 *fumigatus* isolated from rice husk waste dumpsite. *Biosciences* **6** (6):155-159.
342
343 Bassey, N. (2010). *Oil Politics: Nigeria's Unacceptable Biofuels Policy.* Retrieved from
344 http://234next.com/csp/cms/sites/Next/Money/5643461-183/story.csp,on August 15, 2011.
345

UNDER PEER REVIEW

346 Bodirlau, R., Teaca, C. A and Spiridon, .I. (2008)"Chemical modification of beech wood; Effect
347 on thermal stability" *Bioresources,* 3 (3):789-800.
348
349 Cheng, S.W. and Anderson, B.C.(1997). Investigation of ethanol production from municipal
350 primary waste water. *Bioresource Technology*, **59**:81-96.
351
352 Das, H. and Singh, S. K.(2004).Useful byproduct from cellulosic wastes of agriculture and food
353 industry. A Critical appraisal. *Critical Review food science Nutrition* **44** (2):77-89.
354
355 David E., Stephen, D., Helen, A., Rosemary, H. and Robyn, B. (2007).Descriptions of medical
356 fungi. 2$^{nd}$ edition. Australia ,pp100.
357
358 Demain, A., Newcomb, M. and Wu, D. (2005). Cellulase, Clostridia, and Ethanol Microbiology.
359 *Molecular Biology Reviews* **69** (69):124–154.
360
361 Demirbas, A. (2005). Estimating of structural composition of wood and non wood biomass
362 samples. *Energy Sources,* **27**:761-7
363
364 Devanathan, A., Shanmugun, T., Balasubraminian and Manivannan, S. (2007). Cellulase
365 production from coastal mangrove debris. *Trends Applied sciences Research journal,*
366 **2**:23-27.
367
368 Domsch, K. H and Grams, W. (1970). *Fungi in agricultural soils*, Longman group limited.
369 London, pp: 20-152.
370
371 Elijah, A. J., Ojimelukwe, P. C., Ekong, U. S. and Asamudo, N. U.(2010).Effect of *Sacoglottis*
372 *gabonensis* and *Alstonia boonei* on the kinetics of *S.cerevisiae* isolated from palm wine,
373 *African journal Biotechnology* **9** (35):5730-5734.
374
375 Ekunsaumi, T. (2006). *Laboratory production and assay of Amylase by fungi and Bacteria*. Bio-
376 Link Organization, UW-Washington Country.
377
378 Essien, J., Akpan, E., and Essien, E., (2005). Studies on mould growth and biomass production
379 using waste banana peel. *Bioresources Technology*.**96** (13):1451–1456.
380
381 Epstein, J. L., Vieira, M., Aryal, B., Vera, N. and Solis, M.(2010) Developing biofuel in the
382 teaching laboratory: Ethanol from various sources. *Journal Chemistry Education*,**87**
383 (7):708-710
384
385 Flickinger, M. C.(1980). Current biological research in conversion of cellulosic carbohydrate
386 into liquid fuels: how far have we come. *Biotechnology Bioengineering* **22**:27-48.
387
388 Gershon, J. S., Ronald, A. S., Lawrence, B. and Rose, S. B. (1981).Chemical Technicians Ready
389 Handbook, 2$^{nd}$ edition, McGraw-Hillbook company, USA.
390

UNDER PEER REVIEW

391 Goldemberg, J. (2008). In Proceeding of the Conference on Ecological Dimensions of Biofuels.
392 Washigton, DC.
393 http://www.esa.org/biofuel/presentations/Goldemberg_Biofuelspresentation.pdf,27/04/201
394 8
395
396 Hallsworth, J. E., (1998). Ethanol induced water stress in yeast. *Journal of Fermentation and*
397 *Bioengineering,* **85** (2):125-137.
398
399 Huang, X. L., and Penner, M. H., (1991). Apparent substrate inhibition of the *Trichoderma*
400 *reesei* cellulase system. *Journal of Agricultural Food Chemistry.* **39**: 2096–2100.
401
402 Itelima, J., F. Onwuliri,F., Onwuliri, E., Onyimba, I. and Oforji, S.(2013) Bio-Ethanol
403 Production from Banana, Plantain and Pineapple Peels by Simultaneous Saccharification
404 and Fermentation Process. *International Journal of Environmental Science and*
405 *Development.*4:2.
406
407 Jaafaru, M. I. and Fagade O. E. (2007). Cellulase Production and Enzymatic Hydrolysis of Some
408 Selected Local Lignocellulosic Substrates by a strain of *Aspergillus niger. Research*
409 *Journal of Biological Science* **2** (1): 13 – 16.
410
411 Jones R. P. and Greenfield, P.F. (1986). Role of water activity in ethanol fermentation.
412 *Biotechnology and Bioengineering*, **XXVII**: 29 – 40.
413 Kordylas, J. M. (1990). Processing and preservation of tropical and subtropical foods. Macmillan
414 Education Limited, Houndmills pp. 105- 107.
415 Laopaiboon, P., Thani, A., Leelavatcharamas, V., and Laopaiboon, L. (2010). Acid hydrolysis
416 of sugarcane bagasse for lactic acid production. *BioresourceTechnology***101**:1036–1043.
417
418 Mohamed, M. A, and Reddy, C.A. (1986) Direct fermentation of potato starch to ethanol by
419 cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Applied and*
420 *Environmental Microbiology.* 1055-1059.
421
422 Mie-ling, W., Youk-meng , C., Nan-wei, S. and Hsiung, L. (2003). A rapid method for
423 determination of ethanol in alcoholic beverages using capillary gas chromatography.
424 *Journal food drug Analysis*,**11** (2):133-140.
425
426 Miller, G. L.(1959).Use of dinitosalicyclic acid reagent for determination of reducing. *Analytical*
427 *chemistry*, 31:426.
428
429 Ng chiam,Y., Moo-young, J., Jinwon, L. and Min-kyu, O.(2012).Production of 2,3-butanediol in
430 *Saccharomyces cerevisae* by insilico aided metabolic engineering. *Microbial cell*
431 *factories*,11:68.
432
433 Ocloo, F. C. K. and Ayernor, G. S. (2010) Production of alcohol from cassava flour hydrolysate.
434 *Journal of Brewing and Distilling*. **1** (2) :15-21.

UNDER PEER REVIEW

435
436 Oniemola, P .K. and Sanusi, G. (2009). *The Nigerian Bio-Fuel Policy and Incentives* (2007): *A*
437 *Need to Follow the Brazilian Pathway*. International Association for Energy Economics.
438
439
440 Oyeleke, S.B., Dauda, B.E., Oyewole, O.A., Okoliegbe, I.N. and Ojebode, T. 2012. Production
441 of Bioethanol from *Cassava* and Sweet Potato Peels. *Advances in Environmental Biology,*
442 **6** (1): 241-245.
443
444 Oyeleke, S. B. and Manga, S. B. (2008).*Essential laboratory practical in microbiology*, 1st
445 edition. TOBEST publishers, Minna Nigeria. pp 36-37.
446
447 Patel, S. J., Onkarappa, R., and Shoba, K. S. (2007). Fungal Pretreatment Studies on Rice Husks
448 and Bagasse. *Electronic Journal of Environmental, Agricultural, and Food Chemistry ,* **6**
449 (4):1921-1926.
450
451 Penner, M. H., and Liaw, E. T., (1994). Kinetic consequences of high ratios of substrate to
452 enzyme saccharification systems based on Trichoderma cellulase. In: Himmel, M.E.,
453 Baker, J.O., Overend, R.P. (Eds.), Enzymatic Conversion of Biomass for Fuels
454 Production. American Chemical Society, Washington, DC, pp. 363–371.
455
456 Pimentel D., and Patzek, T.W.(2005).“Ethanol Production Using Corn. Switch grass and wood;
457 Biodiesel Production using Soybean and sunflower,” *Natural Resources Research*, **14**
458 (1):65-76.
459
460 Rabah, A. B., Oyeleke, S. B., Manga, S. B. and Hassan, L. G. (2011). Microbial pre treatment of
461 rice husk and groundnut shell for bioethanol production. *International Research Journal*
462 *of Microbiology*, **2 (**8**):** 253-258.
463
464 Reddy, H. K., Srijana M., Madhusudhan, R. D. and Gopal, R., (2010)“Coculture fermentation
465 of banana agro-waste to ethanol by cellulolytic thermophilic *Clostridium thermocellum*
466 CT2”, *African Journal of Biotechnology* , **9** (13): 1926-1934.
467
468 Tchobanoglous, G., Theisen, H., and Vigil, S., (1993). Integrated Solid Waste Management:
469 Engineering Principles and Management Issues. MCGraw-Hill, New York, **3**–22.
470
471 Togarepi, E., Mapiye, C., Muchanyereyi, N. and Dzomba, P. (2012) Optimization of
472 Fermentation Parameters for Ethanol Production from *Ziziphus mauritiana* Fruit Pulp
473 Using *Saccharomyces cerevisiae* (NA33). *International Journal of Biochemistry Research*
474 *& Review* **2 (**2): 60-69.
475
476 Saquido, M. A.P., Cayabyab, V. A and Vyenco, F. R.(1983).Production of microbial protein for
477 feed banana rejects. Natural Science Research Centre, University Of Philippines Quezon
478 City, Philippines, pp 177.
479
480

UNDER PEER REVIEW

481 Ueda, S., Zenin, C.T., Monteiro, D.A. and Park, Y.K. (1981) Production of ethanol from raw cassava starch by a non-conventional fermentation method. In: John Wiley and Sons Inc. *Biotechnology and Bioengineering*, **23**: 291-299.

485 Vallet, C., Said, R., Rabiller, C. and Martin, M. L. (1996). Natural abundance isotopic fractionation in the fermentation reaction: influence of the nature of the yeast. *Bioorganic Chemistry* **24**: 319-330.

489 Wen, Z., Liao W., and Chen S., (2004). Hydrolysis of animal manure lignocellulosic for reducing sugar production. *Bioresources Technology*, **91**:31-39

492 Yabaya, A. and Ado, S. A.(2008) Mycelial protein production by Production by production by *Aspergillus niger* using banana peels, *Science world journal* **3**:9-12

494
495
496
497
498
499
500
501
502
503

UNDER PEER REVIEW