

PROCESS OPTIMIZATION OF BIOETHANOL PRODUCTION FROM PEELS OF DIFFERENT CASSAVA CULTIVARS USING DIFFERENT MICROBIAL INNOCLANTS

by

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**CASSAVA IS CULTIVATED EXTENSIVELY AS
A MAJOR FOOD CROP IN NIGERIA AND OTHER TROPICAL
REGIONS**

**IT IS PROCESSED INTO TAPIOCA, GARRI, FUFU AND
CASSAVA FLOUR**

**IT IS THE WORLD'S LARGEST SOURCE OF CARBOHYDRATE
IN THE WORLD**

**THERE'S A GROWING INTEREST IN THE
CONVERSION OF ORGANIC WASTES LIKE
CASSAVA PEELS INTO USEFUL END-PRODUCTS**


ATTRACTIVE FEATURES OF CASSAVA:

high yielding

able to grow on marginal soils

requires minimal labour and mangt costs

Thus, good candidate for bioethanol prod



**OBJECTIVE OF STUDY - TO OPTIMIZE THE PRODUCTION OF
ETHANOL USING DIFFERENT MICROBIAL INOCULANTS
FOR THE SIMULTANEOUS SACCHARIFICATION AND
FERMENTATION OF CASSAVA PEELS IN 3 CASSAVA
VARIETIES .**



INNOCULANTS USED

- ***Aspergillus niger + Saccharomyces cerevisiae***
- ***Aspergillus niger + Saccharomyces cerevisiae + Rhizopus nigricans***
- ***Spirogyra africana + Saccharomyces cerevisiae + Rhizopus nigricans***
- ***Aspergillus niger + Saccharomyces cerevisiae + Spirogyra africana***
- ***Rhizopus nigricans + Saccharomyces cerevisiae***

METHODOLOGY :

Peels from 3 cassava cultivars TME 0505, TME 41 and TME 4779, were washed, dried in an oven at 60°C for 3 hours, ground into a fine texture using a locally made milling machine & sieved with 1.5µm nylon sieve.

Cultured under anaerobic condition at different concentrations in small bioreactors using the five inoculants separately.


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- **Optical Density (microbial growth) was measured using a spectrophotometer every 3 days.**
 - **Trial fermentation was done to find out the optimal parameters required for high ethanol yields**
 - **Amount of Ethanol produced was recorded at 7, 14 & 21 days using optimal parameters**

TABLE 1

Optical densities (indicating microbial growth) for different inoculum at 640nm and the observed pH (in brackets) in cassava cultivar TME 050

Inoculum	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
<i>Rhizopus/yeast</i>	1.131 (5)	1.22 (4)	1.339 (4)	1.485 (5)	1.33 (5)	1.22 (5)	1.064 (5)
<i>Aspergillus/yeast</i>	0.915 (6)	1.272 (6)	1.535 (5)	1.383 (5)	1.262 (5)	1.255 (5)	1.011 (5)
<i>Aspergillus/ Rhizopus/yeast</i>	1.12 (5)	1.34 (5)	1.401 (5)	1.471 (5)	1.125 (5)	0.95 (6)	1.001 (6)
<i>Rhizopus/Spirogyra/yeast</i>	0.804 (5)	0.914 (5)	1.42 (5)	1.608 (5)	1.632 (5)	1.35 (6)	1.226 (6)
<i>Aspergillus/ Spirogyra/ yeast</i>	0.929 (5)	1.056 (5)	1.614 (5)	1.61 (5)	1.456 (5)	1.232 (6)	1.325 (6)

.D

1.8

1.6

1.4

1.2

1

0.8

0.6

0.4

0.2

0

Day 0

Day 3

Day 6

Day 9

Day 12

Day 15

Day 18

Day 21

Rhizopus/yeast

Rhizopus/Spirogyra/yeast

Aspergillus/yeast

Aspergillus/ Spirogyra/ yeast

Aspergillus/ Rhizopus/yeast

FIG. 1: Growth curve obtained from treating cassava cultivar TME 0505 with different inocula

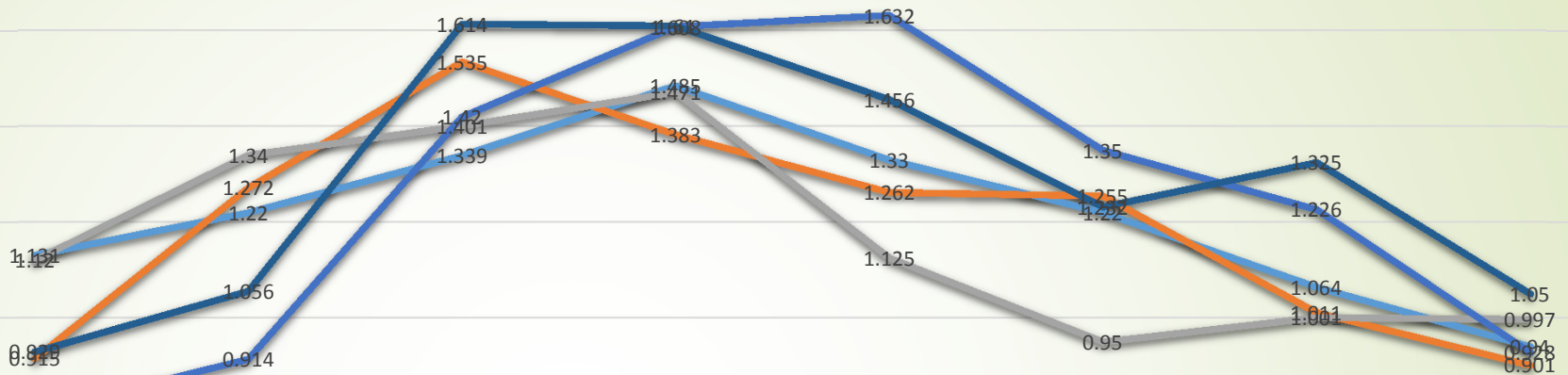


TABLE 2

Microbial growth from different inoculants at optical density of 540nm and the observed pH, in cultivar TME 419

TME 419								
Inoculum	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
<i>Rhizopus/yeast</i>	1.03 (5)	1.362 (4)	1.345 (5)	1.551 (5)	1.194 (5)	1.23 (6)	0.992 (6)	1.011 (6)
<i>Aspergillus/yeast</i>	0.94 (5)	0.997 (4)	1.349 (5)	1.245 (5)	1.139 (5)	1.147 (5)	1.202 (6)	1.101 (6)
<i>Aspergillus/Rhizopus/yeast</i>	0.983 (5)	1.008 (5)	1.42 (5)	1.22 (5)	1.036 (5)	0.995 (6)	1.002 (6)	0.922 (6)
<i>Aspergillus/Spirogyra/yeast</i>	0.801 (6)	1.294 (5)	1.643 (5)	1.62 (5)	1.52 (6)	1.41 (6)	1.229 (6)	1.16 (6)
<i>Aspergillus/ Spirogyra/ yeast</i>	0.903 (5)	1.087 (5)	1.639 (5)	1.598 (5)	1.401 (6)	1.4 (6)	1.34 (6)	1.2 (6)

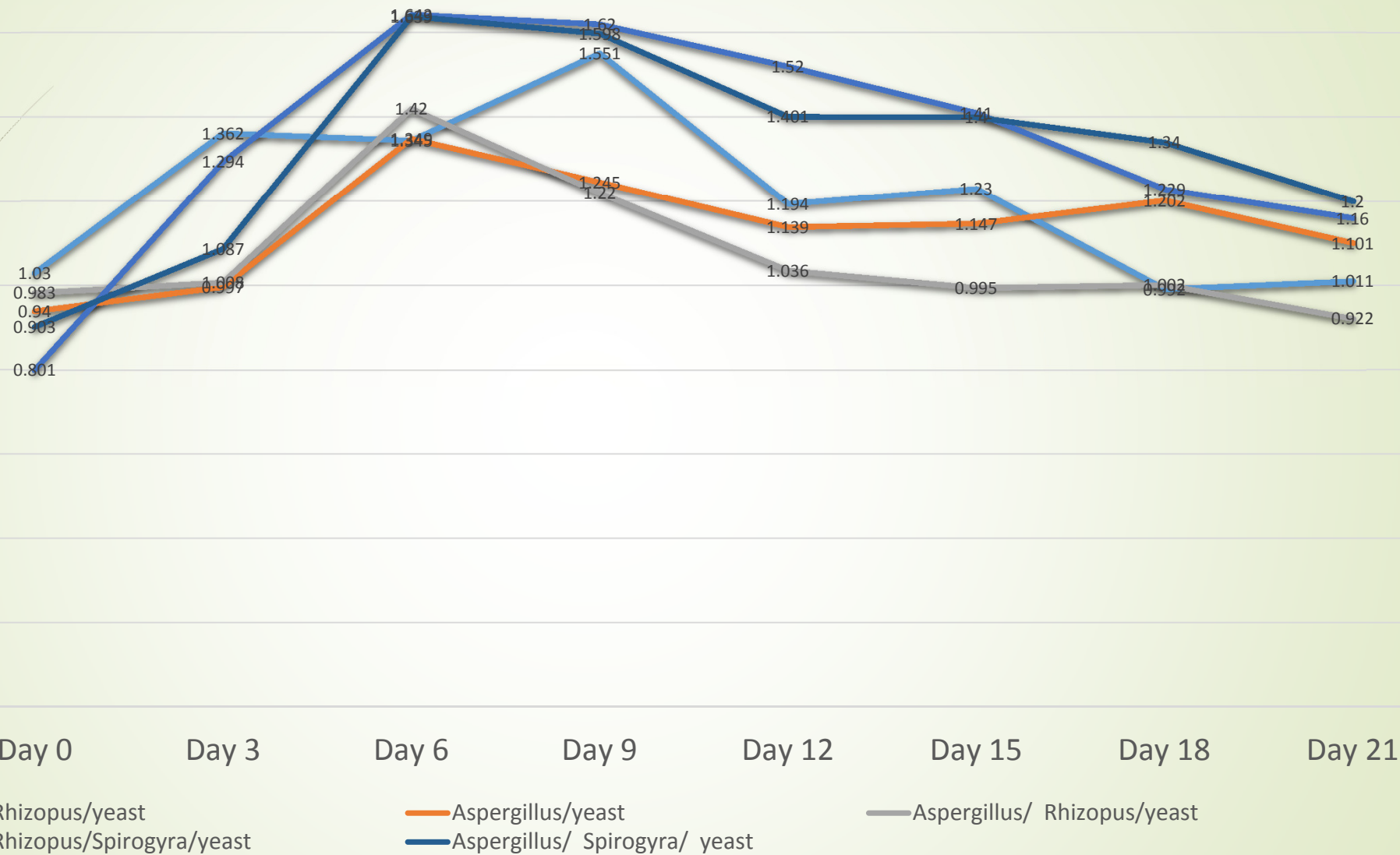


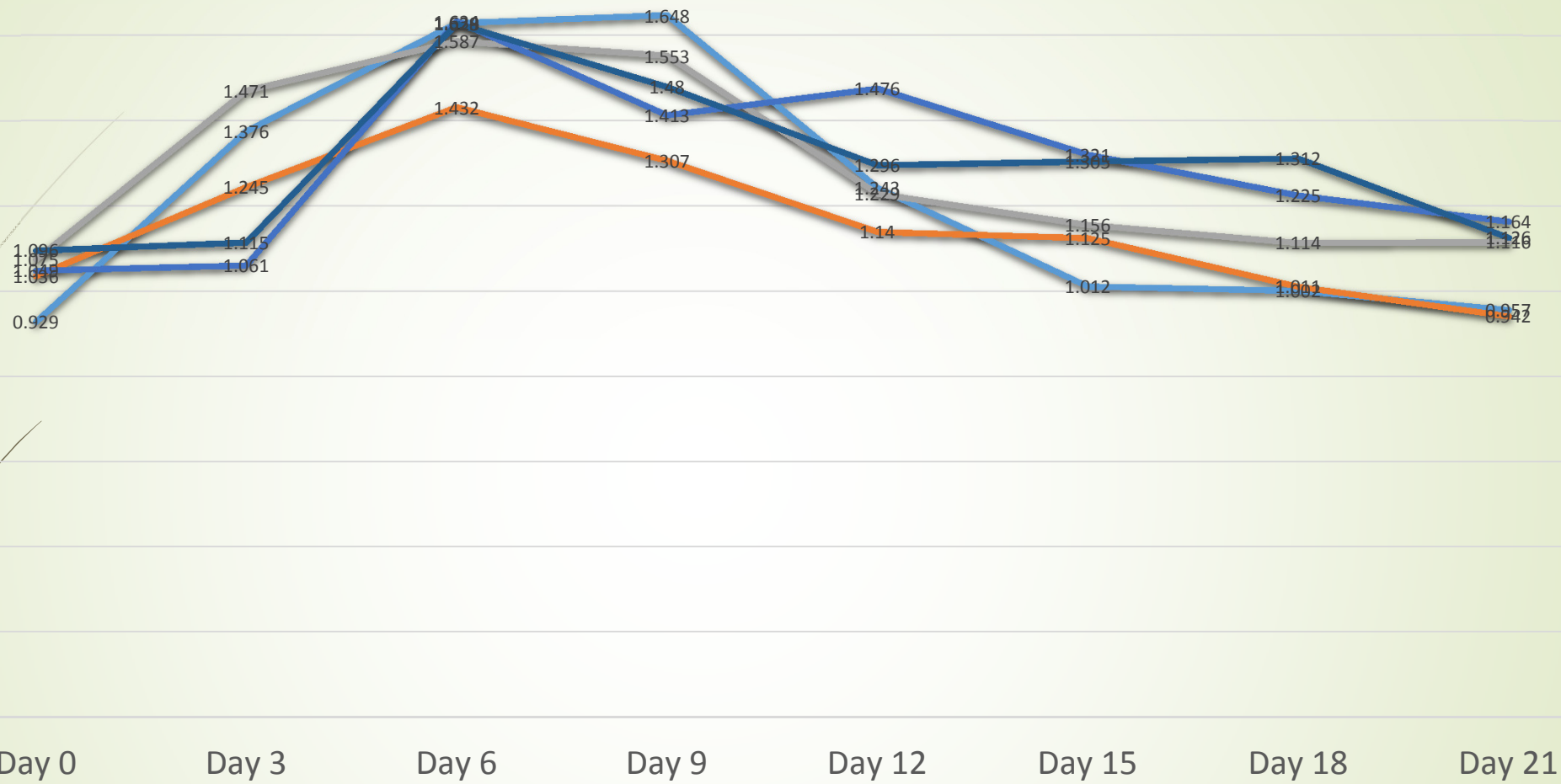
FIG. 2: Growth curve obtained from treating cassava cultivar TME 419 with different inoculants

TABLE 3

Microbial growth for different inoculum at optical density of 540nm and the observed pH, on cassava cultivar TME 4779

TME 4779

Inoculum	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
<i>Rhizopus/yeast</i>	0.929 (4)	1.376 (5)	1.629 (5)	1.648 (5)	1.243 (5)	1.012 (5)	1.002 (5)	0.957 (5)
<i>Aspergillus/yeast</i>	1.036 (5)	1.245 (5)	1.432 (5)	1.307 (4)	1.14 (5)	1.125 (5)	1.011 (6)	0.942 (5)
<i>Aspergillus/Rhizopus/yeast</i>	1.075 (5)	1.471 (5)	1.587 (5)	1.553 (5)	1.229 (5)	1.156 (6)	1.114 (6)	1.116 (5)
<i>Aspergillus/Spirogyra/yeast</i>	1.049 (5)	1.061 (5)	1.634 (5)	1.413 (5)	1.476 (6)	1.321 (6)	1.225 (6)	1.164 (5)
<i>Aspergillus/ Spirogyra/ yeast</i>	1.096 (5)	1.115 (5)	1.628 (5)	1.48 (6)	1.296 (6)	1.305 (6)	1.312 (6)	1.126 (5)



Rhizopus/yeast
 Aspergillus/yeast
 Aspergillus/ Rhizopus/yeast
 Rhizopus/Spirogyra/yeast
 Aspergillus/ Spirogyra/ yeast

3: Growth curve obtained from treating cassava cultivar TME 4779 with different inoculants

RESULTS OF FERMENTATION AT OPTIMIZED PARAMETERS

pH 5.0, temperature 28°C, increased surface area of substrate, substrate concentration and inoculant culture concentration on Days 7, 14 and 21 are shown in tables 5, 6 and 7.

TABLE 5

Ethanol yield obtained on the 7th day from three cassava cultivars treated with the different inoculants

	TME 0505		TME 419		TME 4779	
Cultivar	vol (ml)	mass (g/cm)	vol (ml)	mass (g/cm)	vol (ml)	mass (g/cm)
A	13.33 ^b ± 1.33	10.71 ^b ± 1.07	13.33 ^b ± 0.88	10.71 ^b ± 0.71	17.33 ^b ± 2.94	13.92 ^b ± 0.71
B	13 ^b ± 0.58	10.44 ^b ± 0.47	15.33 ^b ± 0.34	12.32 ^b ± 0.27	16.00 ^b ± 1.00	12.86 ^b ± 0.47
C	13.33 ^b ± 0.34	10.71 ^b ± 0.27	14.66 ^b ± 0.88	11.78 ^b ± 0.71	16.00 ^b ± 0.00	12.85 ^b ± 0.47
D	18 ^a ± 1.00	14.46 ^a ± 0.80	18.33 ^a ± 2.03	14.73 ^a ± 1.63	23.67 ^a ± 0.67	19.01 ^a ± 0.47
E	17.33 ^a ± 0.88	13.92 ^a ± 0.71	18 ^a ± 0.58	14.46 ^a ± 0.47	21.33 ^a ± 0.33	17.14 ^a ± 0.47
Control	8.67 ^c ± 1.20	6.96 ^c ± 0.97	8.67 ^c ± 1.46	6.96 ^c ± 1.17	11 ^c ± 1.00	8.84 ^c ± 0.47

TABLE 6

anol yield obtained on the 14th day from three cassava cultivars treated with different inoculants

Cultivar	TME 0505		TME 419		TME 4779	
	vol (ml)	mass (g/cm)	vol (ml)	mass (g/cm)	vol (ml)	mass (g/cm)
A	12.33 ^b ± 0.88	9.91 ^b ± 0.70	13.67 ^b ± 0.88	10.98 ^b ± 0.71	17 ^b ± 3.22	13.66 ^b ± 2.00
B	17.67 ^b ± 1.20	14.19 ^b ± 0.97	17 ^b ± 1.53	13.67 ^b ± 1.23	16 ^b ± 1.00	12.86 ^b ± 0.88
C	13.67 ^b ± 1.20	10.97 ^b ± 0.97	12.67 ^b ± 0.34	10.17 ^b ± 0.27	14 ^b ± 2.33	11.24 ^b ± 1.00
D	15.67 ^b ± 1.86	12.49 ^b ± 1.46	14.46 ^b ± 1.77	11.78 ^b ± 1.42	21.33 ^b ± 0.88	17.14 ^b ± 1.00
E	19.57^a ± 1.33	15.8^a ± 1.07	19^a ± 1.16	15.26^a ± 0.93	22.33^a ± 0.88	17.94^a ± 0.88
Control	6.67 ^c ± 0.88	5.89 ^c ± 0.97	7 ^c ± 0.58	5.62 ^c ± 0.81	9.33 ^c ± 2.60	9.34 ^c ± 1.00

TABLE 7

Ethanol yield obtained on the 21st day from three cassava cultivars treated with different inoculants

Cultivar	TME 0505		TME 419		TME 4779	
	vol (ml)	mass (g/cm)	vol (ml)	mass (g/cm)	vol (ml)	mass (g/cm)
A	10.66 ^b ± 0.34	8.57 ^b ± 0.27	11.67 ^b ± 1.20	9.37 ^b ± 0.96	15.33 ^b ± 2.33	12.32 ^b ± 1.00
B	14 ^b ± 0.58	11.25 ^b ± 0.47	15.33 ^b ± 1.86	12.31 ^b ± 1.49	13 ^b ± 1.00	10.45 ^b ± 0.58
C	11 ^b ± 0.58	8.84 ^b ± 0.47	11.33 ^b ± 0.33	9.11 ^b ± 0.27	11 ^b ± 0.58	9.37 ^b ± 0.58
D	17 ^a ± 1.53	13.66 ^a ± 1.22	15.67 ^a ± 1.67	12.59 ^a ± 1.34	22 ^a ± 1.73	17.67 ^a ± 1.53
E	21 ^a ± 1.53	16.87 ^a ± 1.22	21.33 ^a ± 0.66	17.14 ^a ± 0.53	21.33 ^a ± 0.34	17.14 ^a ± 0.53
Control	9.67 ^c ± 0.67	7.77 ^c ± 0.54	7.67 ^c ± 0.88	6.16 ^c ± 0.71	10 ^c ± 1.00	8.03 ^c ± 0.58

TABLE 8

Proximate Analysis of Cassava Cultivars

Parameter Tested	TME 4779	TME 419	TME 0505
Moisture Content (%)	62.19	63.59	64.15
Dry Matter (%)	34.92	30.72	25.77
Starch (%)	72.19	70.44	69.85
Fibre (%)	3.42	3.22	3.12
Ash (%)	0.45	0.47	0.28
Protein (%)	2.25	1.15	1.26
Fat (%)	0.61	0.72	0.78
Carbon Nitrogen Phosphorus(ppm)	12.27	6.29	8.56

DISCUSSION

The yield reported in this study competes favorably with (and sometimes better than) those reported from cassava peels using other inoculants, potato peels and spoiled mangoes by other workers.

Growth curves obtained using the five treatments differed slightly, reflecting differences in the enzymatic composition of these microorganisms and in the composition of peels of the three cassava varieties.

An optimum pH of 5.0 obtained in this study is the pH at which each curve showed maximum activity.


Cassava cultivar 4779 when treated with *Rhizopus nigricans*, *Spirogyra africana* and *Saccharomyces cereviceae* gave the highest yield of 19.01g/c and percentage concentration yield of 22% on the 7th day.

This may be attributed to the presence of *Spirogyra* as an additional carbon source for the microorganisms.

Spirogyra generally is known to be autotrophic and its carbohydrate composition can also lead to increase in the release of sugars for fermentation. This result is in line with the work of Sulfahri et al., (2011) but gave a higher yield in the presence of cassava peel substrate and good pH conditions.

ethanol yield obtained in the present study was much lower than the 67.7% and 63.8% reported by Odeleke and Jubril 2009 when *Aspergillus niger* and *Zymomonas mobilis* were used simultaneously on guinea corn husk and millet husk respectively.

In line with reports that *Saccharomyces cerevisiae* is a non- amylolytic microorganism unable to hydrolyse starch (Jumai et al., 2006) low concentrations of ethanol were obtained in this study.



TME 4779 has the highest % starch composition of 72.19, Carbon-Nitrogen-Phosphorus (ppm) of 12.27 and dry matter of 4.92%; this may have contributed to the higher ethanol yield from it. Also the low fiber and ash content of cassava cultivar TME 0505 made it susceptible to quick microbial breakdown.

CONCLUSION


The process of bioethanol production can be optimized for increased ethanol yield at a reduced period of fermentation by improving the various parameters affecting the medium (pH, substrate surface area and concentration).

Bioethanol production from *Spirogyra* holds significant potential due to their low percentage lignin and hemicellulose content compared to other ligno-cellulosic plants.



Spirogyra can be cultivated and proliferate more, as a major feedstock for bioethanol production.

Cassava cultivars can be developed with higher starch content and lower fibre value so that the wastes generated can have high bioethanol potential.



There is need to invest in large-scale biotechnology plants for conversion of biomass (agricultural wastes) to useful products as this will provide wealth and employment.

The use of agricultural wastes instead of staple crops for biofuel production will not infringe on available food supply. It will in addition reduce pollution arising from improper management of these wastes.



**THANKS FOR
YOUR
ATTENTION**