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Solid State Fermentation of Plantain Peels for Bioethanol Production by *Saccharomyces cerevisiae* and *Aspergillus niger*

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Abstract

Plantain peels and other agricultural wastes are in abundance and are a nuisance to the environment. This study evaluated the possibility of ethanol production from plantain peels in solid-state fermentation using naturally occurring fungi and *Saccharomyces cerevisiae*. Naturally occurring fungi were obtained from the plantain peels by solid-state fermentation and were identified with macroscopic and microscopic methods. Proximate composition of the plantain peel was analyzed using standard methods. Co-cultures of isolated fungus and *S. cerevisiae* were adopted for ethanol production in solid-state fermentation for an incubation period of five days. Assay was done daily using the fermented filtrate, which was used to determine the reducing sugar, ethanol yield, total titratable acid, pH, and specific gravity using standard microbiological procedures. The plantain peel has carbohydrate content of 41.66%, protein, 6.98%, and moisture, 7.08%. The highest reducing sugar, 31.20 ± 0.14 mg/L, was obtained on day 1 by co-cultures of *S. cerevisiae* and *A. niger*. The highest ethanol yield, 7.22 ± 0.13 mg/L, was obtained on day 5 by co-cultures of *S. cerevisiae* and *A. niger*. Total titratable acid (TTA) ranged between 33.64 ± 0.06 and 59.04 ± 0.11 mg/L. The pH ranged between 3.91 ± 0.01 and 5.18 ± 0.01. The specific gravity ranged between 0.925 ± 0.06 kg/m^3 and 0.995 ± 0.01 kg/m^3. In conclusion, co-cultures of *S. cerevisiae* and *A. niger* have great potential for bioethanol production using plantain peels in solid-state fermentation.

1.0 Introduction

Bioethanol can be used as a bio-solvent in the laboratory, pharmaceutical, cosmetic, medical, and biomedical industries [1]. It has also been found useful as an alternative fuel for engines. In view of the rising demand for ethanol, there has been increasing interest in searching for alternative sources for its [2]. Biofuel has been gaining momentum in terms of research and development. Since there are various factors, such as the recent rise in oil prices, the increase in demand for fossil fuel, the depletion of mineral oil reserves, and the increase in demand for energy with the increase of the world population and urbanization, The negative impacts of fossil fuel on the environment and the unstable oil market are the major factors that lead to the constant search for alternative fuels [3].

Ethanol, an important biofuel, having a high calorific value, has the added advantage of being less polluting than most sources of energy that are in use today. Previous natural substrates for ethanol production through saccharification include sugarcane bagasse, wheat straw, corn, and softwood [4]. Ethanol is a volatile, flammable, colorless liquid with a light chemical odor. The molecule has an ethyl group linked to a hydroxyl group. The physiochemical properties of ethanol are associated with their chemical structures [1].

Plantains are perennial crops that take the appearance of trees as they mature. Diverse cultivars are grown; *Musa* are believed to have originated in Southeast Asia, but their introduction into Africa is unclear [5]. Throughout history, *Musa* has provided humans with food, medicine, clothing, tools, shelter, furniture, paper, and handicrafts. It could be termed as the "first fruit crop," as its cultivation originated during a time when hunting and gathering were still the principal means of acquiring food [6].

Plantain peels are used as feedstock for cattle, goats, pigs, poultry, rabbits, fish, and several other species [7], as they have some nutritional value; the peels are widely used for that purpose on small farms in regions where plantains are grown. The specific nutrient contained in plantain peels depends on the stage of maturity and the cultivar; for example, plantain peels contain less fiber than dessert plantain peels, and lignin content increases with ripening (from 7 to 15% dry matter). Green plantain peels contain 40% starch that is transformed into sugars after ripening. Plantain peels are also used for ethanol production, cellulase, and as fertilizer and in composting [5].

The process of solid-state fermentation was found to increase the yield of ethanol not only by eliminating inhibition effects but also by eliminating the need for separate reactors for saccharification and fermentation and also reducing economic problems consisting of the cost of production and purification of hydrolytic enzymes [8].

Microorganisms capable of fermenting various sugars and tolerating alcohol and sugar stress are ideal for ethanol production. Examples of such organisms that can ferment various sugars and tolerate alcohol and sugar stress are *Zymomonas mobilis*, *Saccharomyces cerevisiae*, *S. uvarum*, *Candida tropicalis*, *C. shehatae*, *Aspergillus niger*, and *Clostridium* species. Among these organisms, *S. cerevisiae* and *Z. mobilis* stand out as highly efficient microorganisms in the industrial production of alcohol [8]. Although there are reports of bioethanol production through submerged fermentation, this research, however, explored the

possibility of producing bioethanol from plantain peels using *S. cerevisiae* and *A. niger*.

2.0 Materials and Methods

2.1 Collection of Samples

Ripe plantain peels were obtained from roasted plantain roadside vendors at Tipper Garage, Tanke, Ilorin, Kwara State, Nigeria. The ripe plantain peels were chopped into smaller sizes and sundried for fourteen (14) days. The sundried plantain peels were then oven dried in a Nüve FN 120 hot air oven at 40^0C for two (2) hours, after which it were pulverized using an electric blender [5].

2.2 Proximate Analysis of Plantain Peels

The proximate compositions determined include ash content, fiber, lipid, protein, moisture content, and carbohydrates [9].

2.3 Preparation of Culture Medium

Thirty-nine grams of potato dextrose agar (PDA) was weighed, poured into a conical flask, and 100 mL of distilled water was added. The flask was covered with cotton wool and aluminum foil. The solution was dissolved using a Bunsen burner and then sterilized using an autoclave at 121^0C for 15 minutes. After sterilization, the medium was allowed to cool, after which an appropriate volume was dispensed into Petri dishes and allowed to solidify [10].

2.4 Sample Pre-treatment using Acid Hydrolysis

Fifty grams of the processed plantain peels was put in a sterile conical flask, and 500 mL of 1.5% sulfuric acid was introduced into the conical flask. The solution was heated to 160^0C for 30 minutes. The solution was allowed to cool and then filtered using a filter paper to separate the filtrate from the residue. The pH of the residue was brought to 5.0 by the addition of sodium hydroxide to accommodate the growth of the yeast [5], [11].

2.5 Isolation of Naturally Occurring Fungi from Fermented Plantain Peels

Twenty grams of the processed plantain peel was put in a 250 mL beaker, and 20 mL of distilled water was added to it. It was mixed using a glass rod and then covered with aluminum foil. The mixture was kept in a cupboard for five days to allow for natural fermentation [5]. One gram of the fermented substrate was diluted with 9 mL of distilled water in a test tube, and dilutions were made up to 10^{-5}. 0.1 mL from the fifth test tube was plated on Potato Dextrose Agar (PDA) containing 0.1 mL of streptomycin and incubated at 28°C for 48 hours using the spread plate technique. After the incubation period, the fungi were subcultured until pure cultures were obtained. The culture was maintained on potato dextrose agar [12].

2.6 Identification of Fungal Isolate

The fungal isolate was identified macroscopically and microscopically. Physical features of the colonies in the plates were identified. These include the color of the mycelia, the color of the reverse side, and the growth pattern. For microscopic identification, a drop of lactophenol cotton blue was placed on a clean, grease-free slide. A loopful of the fungal mycelia was placed in the drop of lactophenol cotton blue using a sterile wire loop. It was covered with a coverslip and observed under the microscope using 40x objective lenses [13].

2.7 Standardization of Inoculum

The fungal isolate and *S. cerevisiae* were standardized by using 0.5 McFarland standards. The turbidity of a suspension of the fungal isolate and yeast in sterile test tubes was compared with the turbidity of barium sulfate solution in a test tube [14].

2.8 Bioethanol Production using Solid State Fermentation

Fermentation was carried out in 250 mL conical flasks containing 50 g of hydrolyzed substrate and 50 mL of distilled water. The contents were sterilized in an autoclave at 121^0C for 15 minutes. After sterilization, the sterilized substrate was allowed to cool, and 2 mL each of fungal suspension containing $1.5x10^{-8}$ cells/mL of co-cultures of *A. niger* and *S. cerevisiae* and *S. cerevisiae* were aseptically inoculated separately. The flasks were covered with cotton wool and aluminum foil, shaken, and incubated for a period of five days. After every 24 hours, the fermenting medium was withdrawn aseptically, and one gram each of the fermented substrate by co-cultures of *A. niger* and *S. cerevisiae* was dissolved in 50 mL of distilled water in a beaker, mixed, and allowed to stand for 15 minutes before filtering. The filtrate was used to determine the reducing sugar, ethanol yield, total titratable acid, pH, and specific gravity [15].

2.9 Assay for Reducing Sugar

Two mL of the fermented substrate filtrate was put into a test tube; 1 mL of sugar solution and 2 mL of DNSA were added and vortexed. The test tubes were heated in a water bath for 5 minutes, and then 1 mL of 40% potassium sodium tartrate was added. The absorbance was measured in duplicates at 540 nm using the EMC-165-UV Spectrophotometer [16].

2.10 Assay for Bioethanol Yield

5 mL of the fermented substrate solution was put into test tubes, and 1 mL of potassium dichromate reagent was added into each test tube. Then 2 mL of sodium hydroxide solution was added into each test tube. The test tubes were incubated at 500^oC for 30 minutes. The absorbance was measured in duplicates at 600 nm by using a spectrophotometer [17].

2.11 Assay for Total Titratable Acid (TTA)

Ten mL each of the fermented substrate solution was transferred into a beaker, followed by two drops of phenolphthalein indicator. The solution was then titrated against 0.1 M sodium hydroxide to an end point of a definite pink color [18].

2.12 Assay for pH

The pH of each fermented substrate was determined by using a pH meter (Hanna pH meter model: HI-1285-5). The pH meter was calibrated with a phosphate buffer before use. Five mL of each solution was pipetted into a beaker, and the probe was dipped into it. The pH values were read and recorded in duplicates [11].

2.13 Assay for Specific Gravity

This was done using the refractometer method (refractometer model: refractometer RF 110). The refractometer was calibrated by opening the cover plate, and 2 drops of distilled water were added using a dropping pipette on the prism. The cover plate was closed, pressed lightly, rotated, and the calibration screw was adjusted to make the

light/blue boundary (made up of the brick level and specific gravity scale) with the null line. The cover plate was opened and the surface of the prism was cleaned with a piece of cotton wool. Two drops of the solution to be measured were dropped on the prism, the cover plate was covered, pressed lightly, and the corresponding scale of the light/blue boundary was read and recorded according to AOAC, 2019 [11].

2.14 Statistical Analysis

The data obtained was subjected to one-way ANOVA using the Duncan Homogeneity test. Values with $p < 0.05$ were considered significant. Statistical Package for Social Sciences (SPSS version 26) was used to carry out the analysis.

3.0 Results

3.1 Proximate Compositions of Plantain Peel

The proximate results of the plantain peel indicated that crude lipid (%) was the lowest (4.42), while carbohydrate (%) had the highest value (41.66), as shown in Figure 1. Plantain peels are primarily composed of carbohydrates with minimal lipid content, making them ideal for ethanol production. Their high sugar content and low lipid levels facilitate efficient fermentation, reducing fungal inhibition.

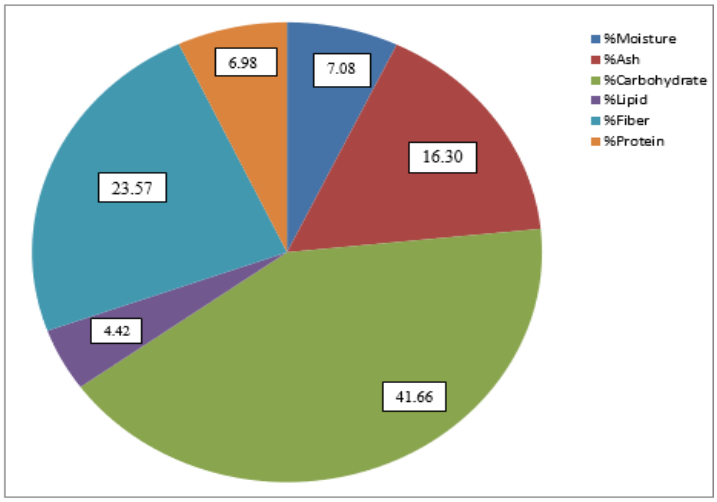


Figure 1: Proximate Composition of Plantain Peel

3.2 Characteristics of Fungal Isolate and Control

The isolates exhibited diverse colonial morphological properties and microscopic characteristics. *Aspergillus niger* had black spore coloration, the presence of spherical conidia with roughened conidiophores, and mycelia with whitish edges. *Saccharomyces cerevisiae* had a creamy color of colonies and oval-shaped cells, as shown in Table 1. They were easily isolated due to their natural abundance in organic materials like soil, air, and decaying matter.

Table 1: Characterization of Fungal Isolates

Fungal Isolates	Macroscopic and Microscopic description	Probable Organisms
F_1	Black spores and mycelia with whitish edges; rough, spherical conidia and conidiophore	*Aspergillus niger*
F_2	Creamy colonies; oval-shaped cell	*Saccharomyces cerevisiae*

Keys: F_1, F_2 = Fungal Isolates

3.3 Reducing Sugar Content during Fermentation by *S. cerevisiae* and Co-Cultures of *S. cerevisiae* and *A. niger*

Over the five days, both cultures exhibited a significant ($p<0.05$) decreasing trend in reducing sugar content (mg/L). The concentration at day one to five showed a significant decrease in *S. cerevisiae* compared to the substrate with co-culture substrate (Table 2). The steady decline in reducing sugar concentration over five days is due to fungal enzyme production breaking down plantain peel carbohydrates into simple sugars for consumption and energy. As fungi ferment these sugars, they produce ethanol and carbon dioxide, reducing sugar content.

Table 2: Reducing Sugars Content during Fermentation by *S. cerevisiae* and Co-Culture of *S. cerevisiae* and *A. niger*

Fermentation period (Days)	*S. cerevisiae* (mg/L)	Co-culture of *S.cerevisiae* and *A. niger* (mg/L)
1	28.90 ± 0.35^{a}	31.20 ± 0.14^{a}
2	26.50 ± 0.28^{b}	27.30 ± 0.31^{b}
3	18.20 ± 0.51^{c}	21.80 ± 0.17^{c}
4	13.70 ± 0.24^{d}	16.40 ± 0.14^{d}
5	10.40 ± 0.69^{e}	11.70 ± 0.11^{e}

Values are means (n=2) ± SD. Values within rows bearing the same superscript are not significantly different from each other.

3.4 Ethanol Yield of Fermented Plantain Peel by *S. cerevisiae* and Co-Cultures of *S. cerevisiae* and *A. niger*

Over the five days, both cultures exhibited a significant ($p<0.05$) increasing trend in bioethanol content (mg/L). The concentration at day one to five showed a significant increase in co-culture substrate compared to substrate with *S. cerevisiae* (Table 3). Ethanol yield increased steadily as reducing sugar levels decreased during incubation, indicating efficient conversion of sugars into ethanol.

Table 3: Bioethanol Content during Fermentation by *S. cerevisiae* and Co-Culture of *S. cerevisiae* and *A. niger* (mg/L)

Fermentation period (Days)	*S. cerevisiae* (mg/L)	Co-culture of *S. cerevisiae* and *A. niger* (mg/L)
1	3.48 ± 0.37^{d}	3.62 ± 0.31^{d}
2	$4.01 \pm 0.61^{c,d}$	4.87 ± 0.38^{c}
3	$5.26 \pm 0.16^{b,c}$	6.03 ± 0.33^{b}
4	$5.93 \pm 0.96^{a,b}$	$6.81 \pm 0.37^{a,b}$
5	6.93 ± 0.01^{a}	7.22 ± 0.13^{a}

Values are means (n=2) ± SD. Values within rows bearing the same superscript are not significantly different from each other.

3.5 Total Titratable Acid (TTA) of Fermented Plantain Peel by *S. cerevisiae* and Co-cultures of *S. cerevisiae* and *A. niger*

Over the five days, both cultures exhibited a significant ($p<0.05$) increasing trend in the total titratable acidity (mg/L). The concentration at day one to four showed a significant increase in co-culture substrate compared to substrate with *S. cerevisiae* (Table 4). This rise is attributed to fungal metabolism, where organic acids are produced as by-products of sugar fermentation.

Table 4: Total Titratable Acidity (TTA) of Plantain Peels during Fermentation by *S. cerevisiae* and Co-culture of *S. cerevisiae* and *A. niger* (mg/L)

Fermentation Period (Days)	Substrate with Co-Culture of *S. cerevisiae* and *A. niger* (mg/L)	Substrate with *S. cerevisiae* (mg/L)
1	35.64 ± 0.23^{e}	33.64 ± 0.06^{e}
2	42.04 ± 0.52^{d}	39.24 ± 0.06^{d}
3	48.84 ± 0.13^{c}	41.84 ± 0.33^{c}
4	50.40 ± 0.40^{b}	48.24 ± 0.41^{b}
5	57.24 ± 0.20^{a}	59.04 ± 0.11^{a}

Values are means (n=2) ± SD. Values within columns bearing the same superscript are not significantly different from each other.

3.6 pH of Fermented Plantain Peel by *S. cerevisiae* and Co-Cultures of *S. cerevisiae* and *A. niger*

Over the five days, both cultures exhibited a significant ($p<0.05$) decreasing trend in pH. The pH at day one to five showed a significant decrease in co-culture substrate compared to substrate with *S. cerevisiae* (Table 5). This can be attributed to acid accumulation during fermentation.

Table 5: pH of Fermented Plantain Peels during Fermentation by *S. cerevisiae* and Co-Culture of *S. cerevisiae* and *A. niger*

Fermentation Period (Days)	pH of Substrate with Co-Culture of *S. cerevisiae* and *A. niger*	pH of Substrate with *S. cerevisiae*
1	5.18 ± 0.01^{a}	5.61 ± 0.01^{a}
2	4.92 ± 0.01^{b}	5.37 ± 0.01^{b}
3	4.53 ± 0.01^{c}	4.92 ± 0.01^{c}
4	4.07 ± 0.01^{d}	4.39 ± 0.01^{d}
5	3.91 ± 0.01^{e}	4.06 ± 0.01^{e}

Values are means (n=2) ± SD. Values within columns bearing the same superscript are not significantly different from each other.

3.7 Specific Gravity of Fermented Plantain Peel by *S. cerevisiae* and Co-Cultures of *S. cerevisiae* and *A. niger*

Over the five days, both cultures exhibited a significant ($p<0.05$) decreasing trend in specific gravity (kg/m^3). The specific gravity

at day one to five showed a significant decrease in co-culture substrate compared to substrate with *S. cerevisiae* (Table 5). This is likely due to the conversion of solids into ethanol, which is less dense than water. Additionally, water absorption by plantain peels may have contributed to the decreased density of the fermentation medium.

Table 6: Specific Gravity of Plantain Peels during Fermentation by *S. cerevisiae* and Co-Culture of *S. cerevisiae* and *A. niger* (kgm^{-3})

Fermentation Period (Days)	Substrate with Co-Culture of *S. cerevisiae* and *A. niger* (kgm^{-3})	Substrate with *S. cerevisiae* (kgm^{-3})
1	0.981 ± 0.03^{a}	0.995 ± 0.01^{a}
2	0.975 ± 0.04^{a}	0.983 ± 0.03^{a}
3	0.970 ± 0.04^{a}	0.977 ± 0.04^{a}
4	0.948 ± 0.05a	0.965 ± 0.04^{a}
5	0.925 ± 0.06^{a}	0.941 ± 0.05^{a}

Values are means (n=2) ± SD. Values within columns bearing the same superscript are not significantly different from each other.

4.0 Discussion

Bioethanol, due to its wide application and safe use to curb environmental pollution, remains in high demand [2]. This study therefore investigated the production of bioethanol from plantain peels using co-cultures of *S. cerevisiae* and *A. niger* and *S. cerevisiae* only in solid-state fermentation.

The successful isolation of *S. cerevisiae* is due to its natural occurrence in the environment, particularly in organic materials like plant matter. This fungus is commonly found in soil, air, and decaying matter, and plantain peel provides a suitable environment for its growth due to its nutrient-rich composition, including carbohydrates and proteins that serve as a food source.

The proximate composition of the plantain peel samples, as shown in Figure 1, indicates that plantain peels are predominantly rich in carbohydrates and have relatively low lipid content. The high carbohydrate content and relatively low lipid content make it suitable for the production of ethanol because a high amount of sugar is needed to produce ethanol, and the low lipid content helps reduce the risk of fungi inhibiting each other during fermentation. This finding corroborates with the findings of Ogunsuyi and Olawale [5], Barnabas and Anthony [19], and Obasanmi *et al*. [20].

The steady decrease in reducing sugar concentration over the five-day period for both cultures can be attributed to several factors, such as enzyme production, which breaks down complex carbohydrates in plantain peel into simpler sugars, which are then consumed by the fungi as a carbon source for growth and energy. As fungi ferment sugars, they convert them into ethanol and carbon dioxide; this reduces the sugar content in the medium. This result agrees with the findings of Adedayo *et al*. [11] and Ollo *et al*. [21].

Throughout the incubation period, the ethanol yield increased as the reducing sugar decreased. This could be attributed to the readily available reducing sugar being converted into ethanol. The high ethanol yield achieved by the co-cultures of *A. niger* and *S. cerevisiae* can be credited to their synergistic properties. This discovery aligns with previous studies by Remsion and Odion [22] and Kitson-Hytey *et al*. [7]. In contrast, Adedayo *et al*. [11] reported a different trend where the ethanol yield peaked at 96 hours and declined at 120 hours during fermentation using *Gmelina arborea* as a substrate.

The total titratable acid (TTA) increased throughout the incubation period and was highest at day 5 for both co-cultures of *S. cerevisiae* and *A. niger* and *S.* cerevisiae. This can be attributed to fungal activities whereby organic acids are released as by-products of sugar metabolism during the fermentation process. This finding agrees with the findings of Audu *et al*. [23], who worked on the production of bioethanol using rice husks.

The decrease in pH may be due to the accumulation of acids being produced during the course of the fermentation. This result agrees with the findings of Ike *et al*. [24], who recorded a decrease in the pH throughout the 8-day fermentation period of plantain and banana peels. However, this result does not agree with the findings of Adedayo *et al.* [11], where the pH fluctuated during the 120-hour fermentation period of *Gmelina arborea* wood waste.

The depletion in the specific gravity followed the same pattern as reducing sugar. This may be due to the conversion of the total solids into ethanol, which is less dense than water, causing a decrease in specific gravity; also, it could be as a result of absorption of water by plantain peels, which reduced the density of the fermentation medium [1]. This finding agrees with the findings of Adedayo *et al*. [11], in which the sugar level and specific gravity decreased throughout the fermentation period of *Gmelina arborea* wood waste.

5.0 Conclusion

This study has demonstrated that plantain peels are suitable substrates for the production of bioethanol using both yeast (*S. cerevisiae*) and filamentous fungi (*A. niger*) in solid-state fermentation. It also demonstrated that using co-cultures of *S. cerevisiae* and *A. niger* produces a higher ethanol yield than using *S. cerevisiae* only, due to the synergism between the two fungi, where *A. niger* hydrolyzes the complex sugars and *S. cerevisiae* ferments the sugars.

Declarations

Consent for Publication

Not Applicable

Availability of Data and Material

The datasets used and/or analysed during the current are available from the corresponding author on reasonable request.

Competing Interest

The authors declare that they have no competing interest

Author's Contribution

AE conceived the idea, supervised the research work, and edited the manuscript; NI carried out the research, collected the data, and analyzed it, while TE wrote the manuscript.

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Not applicable

References

[1] Timothy, T.J., Wiens, D.J. and Reaney, M.J.T. (2021). Production of Bioethanol-A review of factors affecting ethanol yield. *MDPI*. 7(4):268.

[2] Palupi, B., Fachri, B., Rahmawati, I., Susanti, A., Arie, S. F., Adinurani, P. and Mel, M. (2020). Bioethanol used as topical antiseptics: Pretreatment optimization of bioethanol production from tobacco industrial waste. *Annals of Tropical Medicine and Public Health*. 23(S8):1213-1219.

[3] Jun, H., Ke, C., Jingsha, X. and Yong, S. (2024). Urban Air Pollution and Control, Encyclopedia of Sustainable Technologies (Second Edition). 2:143-159
.

[4] Yesmin, M.N., Azad, M., Kamruzzaman, M. and Uddin, M.N. (2020). Bioethanol Production from Corn, Pumpkin and Carrot of Bangladesh as Renewable Source using Yeast *Saccharomyces cerevisiae*. *Acta Chemica Malaysia*. 4(2):45-54.

[5] Ogunsuyi, H.O. and Olawale, C.A. (2021). Evaluation of Plantain Biomass (*Musa paradisiaca*) as feedstock for bioethanol production. *Scientific Research*. 11(2): 59-71.

[6] Uzairu, S. and Atiku, M. K. (2021). African Journal of Food Science Assessment of phytochemical and mineral composition of unripe and ripe plantain (*Musa paradisiaca*) peels. *African Journal of Food Science*. 15(3):107-112.

[7] Kitson-Hytey, M., Fei-Baffoe, B., Sackey, L. and Miezah, K. (2022). Production of bioethanol from plantain and yam peels using *Aspergillus niger* and *Saccharomyces cerevisiae. Biomass Conversion and Biorefinery.* 14(9):1-9.

[8] Edeh, I. (2020). Bioethanol Production: An Overview. *Intechopen.* pp 1-22.

[9] Association of Official Analytical Chemists (2020). Determination of proximate analysis of food sample. *Official Method of Analysis*. 21st Edition.

[10] Pratik, J., Harshajit, N., Puja, B.K., Shivani, D., Padmaja, M., Jayashankar, D. and Sushma, D. (2021). Mycogenic Fabrication of Nanaoparticles and their Appplications In Modern Agricultural Practices & Food Industries. 3:475-488.

[11] Adedayo, M.R., Ajiboye, A.E. and Yahaya, O.A. (2020). Ethanol production from *Gmelina arborea* wood waste by *S. cerevisiae* using solid state fermentation. *Nigeria Journal of Biotechnology.* 37(2): 144-151.

[12] Alonso-Gómez, A. L., Solarte-Tot, J. C. and Cardona-Alzate, C.A. (2020). Performance evaluation and economic analysis of the bioethanol and flour production using rejected unripe plantain fruits (*Musa paradisiaca* L.) as raw material. *Food and Bioproducts Processing*, 121:29-42.

[13] Ezeonuegbu, B.A., Abdullahi, M.D., Whong, C.M.Z, Sohunago, J.W., Kassem, H.S., Yaro, C.A., Hetta, H.F., Mostafa-Hedeab, G., Zouganelis, G.D. and Batiha, G.E. (2022). Characterization and Phylogeny of Fungi Isolated from Industrial Wastewater using Multiple Genes. 12(10:2094.

[14] Souza, F., Gomes, V. and Bitencourt, T. (2020). Use of McFarland Standards and Spectrophotometry for *Yarrowia lipolytica* QU69 cell counting. *International Journal of Environment, Agriculture and Biotechnology*. 5(4):1089-1091.

[15] Ching, A.L., Cheng, C., Li, W.C., Will, C.C. and Kow, J.D. (2023). Ethanol production using the whole solid-state fermented sugarcane bagasse cultivated by *Trichoderma reesei* RUT-C30 supplemented with commercial cellulose. *Biocatalysis and Agricultural Biotechnology*, 50(2):1-7.

[16] Jagessar, R.C. and Garraway, R. (2023). The production of bioethanol from mango (*Mangifera indica*) peel and plantain (*Musa paradisiaca*) peel via water pretreatment, dilute acid hydrolysis and fermentation supported by *Saccharomyces cerevisiae*. *American Journal of Research Communication*. 11(4):1-21.

[17] Marcos, B. C. and Rosana, C. (2022). Spectrophotometric Assay for the Quantification of Plasma Ethanol Levels in Mice through Chromium-Ethanol Oxidation-Reduction Reaction, *Advances in Bioscience and Biotechnology*. 13(4):175-187.

[18] Paul, N. A., Chinedu, P.N., Emmanuel, N.U., Ude, E.I., Elsa, M.S., Badatu, M.M. and Bernadette, H.B. (2023). Effect of fermentation time on nutritional, antinutritional factors and in-vitro protein digestibility of *Macrotermes nigeriensis*-cassava mahewu. *Measurement: Food*. 11

[19] Barnabas, O.O. and Anthony, J.F. (2020). Comparative Evaluation of the Nutritive, Mineral, and Antinutritive Composition of *Musa sinensis* L. (Banana) and *Musa paradisiaca* L. (Plantain) Fruit Compartments. *Plants (Basel).* 8(12):598.

[20] Obasanmi, T.E., Oluwafemi, A.G., Adeyemi, O.S. and Oluba, O.M. (2024). Comparative Evaluation of Peeled and Unpeeled Unripe Plantain on Liver Function Indices and Oxidative Stress Markers in Wistar Rats. *Journal of Harbin Engineering University.* 45(3):119-126.

[22] Remsion, A. and Odion-Owase, A.O. (2019). Production of Ethanol from Plantain, Pineapple and Banana Wastes by Fermentation Process. *Global Scientific Journals.* 7(7):389-397.

[23]Audu, R.O., Ijah, U.J.J. and Mohammed, S.S.D. (2023). Pre-treatment, Physicochemical Properties and Production of Bioethanol from Rice Husk using Fungi Isolated from Waste Dumpsite in Kaduna, *J. Appl. Sci. Environ. Manage*. 27(7):1359-1370.

[24] Ike, C., Onwuakor, C., Kalu, A. and Chukwuma, N. (2019). Citric acid production by *Aspergillus niger* using banana and plantain peels. GSC *Biological and Pharmaceutical Sciences.* 8(2):015-021.