



Investigating the Fungal and Physicochemical Parameters During the Fermentation of Plantain (*Musa paradisiaca*) Peels to Bioethanol

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Abstract: The growing reliance on fossil fuels has led to numerous environmental challenges and exacerbated climate change, underscoring the need for alternative, affordable, renewable, and eco-friendly bioenergy sources. This study examined the fungal and physicochemical parameters involved in the fermentation of bioethanol from plantain (*Musa paradisiaca*) peels in Bayelsa State, Nigeria. *Musa paradisiaca* peels were selected for this research due to their availability in large quantities as waste products in the region. The fermentation process was carried out in two setups: one using *A. niger* and the other using *S. cerevisiae*. Over a 12-day fermentation period, bioethanol was recovered via distillation on the 13th day. The fermentation process was closely monitored, with fungal and physicochemical parameters (temperature, pH, specific gravity, and alcohol content) analyzed using standard methods. Results indicated an increase in total fungal count from 3.1×10^2 to 3.5×10^2 CFU/mL from days 1 to 3, followed by a decrease from 3.5×10^2 to 0 CFU/mL from day 3 to the end of distillation for the setup fermented by *Aspergillus niger*. Similarly, the fungal count for the *Saccharomyces cerevisiae* setup increased from 3.2×10^2 to 3.5×10^2 CFU/mL from days 1 to 3 and then decreased from 3.5×10^2 to 0 CFU/mL after distillation. Throughout the fermentation, the temperature rose from 25.00°C to 30.35°C, while the pH decreased from 5.50 to 3.85. Specific gravity decreased from 1.018 to 0.436, resulting in an increase in alcohol content from 1.50% to 45.00% for the *Aspergillus niger* setup and from 1.60% to 50.00% for the *S. cerevisiae* setup. This study demonstrates that bioethanol can be effectively produced from *Musa paradisiaca* peels using *A. niger* and *S. cerevisiae* as fermenting agents.

Keywords: Bioethanol, Fermentation, Plantain (*Musa paradisiaca*) peels, *Aspergillus niger*

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Introduction

Ethanol is a colourless liquid that is volatile and flammable, characterised by a faint chemical smell. Its molecular structure consists of an ethyl group attached to a hydroxyl group. The chemical structure of ethanol determines its physicochemical properties (Timothy *et al.*, 2021). As a significant biofuel with a high energy content, ethanol offers the benefit of producing less pollution compared to many current energy sources. Natural materials previously used for ethanol production via saccharification include sugarcane bagasse, wheat straw, corn, and softwood (Yesmin *et al.*, 2020).

Research and development in biofuels have been accelerating. Several factors contribute to this trend, including the recent surge in oil prices, growing demand for fossil fuels, depletion of mineral oil reserves, and rising energy needs due to global population growth and urbanisation. Additionally, the environmental harm caused by fossil fuels and the volatility of the oil market are key drivers behind the ongoing pursuit of alternative energy sources (Jun *et al.*, 2024).

At present, the production of bioethanol from sustainable sources like lignocellulosic biomass (LCB), sugar, and starch materials is gaining significant attention as an alternative. The transportation sector is a major source of environmental pollution and high energy use. By 2040, it is projected that this sector will consume about 61% of the world's total oil, contributing to one-fifth of global CO₂ emissions (González-Montaña, 2016). Bioethanol is regarded as an important renewable bioenergy alternative to reduce reliance on fossil fuels. Unlike nonrenewable fossil fuels, bioethanol is produced through the fermentation of sugars under controlled conditions.

The use of waste from plantain and banana plant biomass (PBB) contributes to creating a renewable, appropriate, and environmentally friendly energy source. Alzate Acevedo *et al.* (2021) reported that nearly 60% of plantain biomass is wasted after harvest, totalling about 114.08 million metric tons globally. Approximately 18% to 20% of plantain peels are discarded annually, causing environmental harm (Guerrero *et al.*, 2016). Poor management and

utilisation of plantain and banana plant biomass can lead to increased greenhouse gas emissions, contributing to global warming (PFPI, 2011). Plantain and banana plant biomass is rich in industrial raw materials such as starch, cellulose, hemicellulose, and abundant fibres, which can be converted into valuable products through techniques like pyrolysis, anaerobic digestion (AD), and fermentation.

Different species of plantains contain specific amounts of starch. As the ripening process occurs, starch breaks down into sugars, with ripe bananas having approximately 70% more glucose and fructose compared to fully ripe bananas. Cellulose, a significant component, is mainly utilized as organic matter in excipient production. Being a polymer of glucose, cellulose can be hydrolysed into simple sugars, which can then be fermented to produce bioethanol (Chang *et al.*, 2018).

Plantain peels serve as feed for various animals, including cattle, goats, pigs, poultry, rabbits, and fish due to their nutritional value (Kitson-Hyter *et al.*, 2022). They are commonly used on small farms in plantain-growing regions for this purpose. The nutrient content of plantain peels varies depending on their maturity stage and cultivar. Green plantain peels contain about 40% starch, which converts to sugars during ripening. Additionally, plantain peels are utilized for ethanol production, cellulase enzyme extraction, as fertilizer, and in composting (Ogunsuyi and Olawale, 2021).

Microorganisms that can ferment a variety of sugars while withstanding alcohol and sugar stress are ideal for ethanol production. Examples include *Zymomonas mobilis*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Candida tropicalis*, *Candida shehatae*, *Aspergillus niger*, and *Clostridium* species. Among these, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are particularly notable for their high efficiency in industrial alcohol production (Edeh, 2020). This study aimed to investigate the fungal activity and physicochemical characteristics during the fermentation of plantain (*Musa paradisiaca*) peels into bioethanol in Bayelsa State, Nigeria.

Materials and Methods

Sample Collection: Freshly harvested plantain (*Musa paradisiaca*) was obtained from Swali market, Yenagoa Local Government Area, Bayelsa State, Nigeria. The plantain was transported to the Department of Microbiology laboratory, Federal University Otuoke in sterile containers for further analysis.

Sample Preparation: The *Musa paradisiaca* was peeled, and the peels were sliced into smaller pieces

using a sterile knife and then sun-dried for 5 days. They were then oven-dried for 48 hours at 70 °C to reduce the moisture content to a minimum of 10–12 %. The dried peel slices were ground using a laboratory blender to make fine flour, and the flour was kept in an airtight container under room temperature (Gebregergs *et al.*, 2016).

One hundred (100) grams of the flour was mixed with water in the ratio of 1:5w/v for flour and water, respectively, to make a slurry. The slurry was allowed to undergo saccharification by acid-enzyme hydrolysis. It was treated with ammonium chloride and amylase enzyme (Tibolla *et al.*, 2016). *A. niger* was isolated from a soil sample, and *Saccharomyces cerevisiae* was isolated from the *Musa paradisiaca* peels using standard methods. They were characterised and tentatively identified using morphological and biochemical methods described by Oyeleke *et al.* (2012).

Production of Bioethanol: The fermentation process was carried out by the method described by Ching *et al.* (2023) with slight modifications. The fermentation process was carried out in two (2) different 500 mL conical flasks containing 100g of the hydrolysed substrate and 100 mL of distilled water each. The two (2) conical flasks were used to constitute two (2) setups: one fermented by *A. niger* and another fermented by *Saccharomyces cerevisiae*. The inoculum containing the fungal isolates was aseptically inoculated separately into the two 2 conical flasks and covered with cotton wool and aluminium foil. It was allowed to ferment for twelve (12) days, and aliquots of the sample in the fermenting medium for both setups were taken at an interval of 48 hours to monitor the process.

Fungal Analyses: The fungal count for the two fermenting setups was determined by serially diluting the fermenting slurry, and a dilution factor of 10^{-2} was inoculated onto potato dextrose agar supplemented with chloramphenicol and incubated for 72 hours. This was done in duplicates, after which the colonies were enumerated. The procedure was carried out at an interval of 48 hours for twelve (12) days.

Physicochemical analyses of the bioethanol: The physicochemical parameters, which include the temperature, pH, specific gravity, and alcohol content, were monitored at an interval of 48 hours for twelve (12) days.

Determination of Temperature: The temperature was determined using the method described by Opara and Alabere (2024). Ten (10) mL of the *Musa paradisiaca* peels slurry was collected from the fermenter and placed into a sterile beaker. A

laboratory mercury bulb thermometer was inserted into the beaker, and the readings of the temperature in the course of the fermentation were recorded in duplicates in °C.

Determination of pH: The pH was determined by the method described by Ochai and Kolhatkar (2008). Ten (10) mL of the *Musa paradisiaca* peels slurry was collected from the fermenter at an interval of 48 hours. It was put into a sterile beaker, and a digital pH meter calibrated with standard buffers (pH 4 and 7) was inserted into the beaker to measure the pH. This procedure was done in duplicates.

Determination of Specific Gravity: The specific gravity was determined by the method described by AOAC (2023). Fifty (50) mL specific gravity bottle cleaned with distilled water, oven dried and allowed to cool. The weight of the cool, dried, empty bottle was recorded as W_1 . The dried bottle was filled with deionised water and was weighed (W_2). The specific gravity bottle was emptied and cleaned using the fermenting *Musa paradisiaca* peels slurry, and thereafter the bottle was filled to the brim with the *Musa paradisiaca* peels slurry and weighed (W_3). The specific gravity was calculated as:

$$\text{Specific gravity (SG)} = \frac{S (W_3 - W_1)}{G (W_2 - W_1)}$$

Where S = Weight of the volume of the sample

W = Weight of the volume of water

Determination of Alcohol Content: The alcohol content was determined by the method described by Opara and Alabere (2024). Ten (10) mL of the fermenting slurry was collected from the fermenter and placed into a sterile beaker. An alcohol meter was inserted into the beaker, and the alcohol content was measured in percentage (%) in duplicates.

Recovery of Bioethanol: At the end of the fermentation process, the bioethanol produced was recovered using the procedure described by Oyeleke *et al.* (2012). The bioethanol produced was recovered by distillation. The fermented liquid was transferred into a round-bottom flask and placed on a heating mantle fixed to a distillation column enclosed in a running tap water. Another flask was fixed to the other end of the distillation column to collect the distillate at 78 °C.

Statistical Analysis: The data obtained were statistically analyzed using Student's paired T-test.

Results

Table 1 presents the results of the change in fungal count during the fermentation of bioethanol by *A.*

niger and *Saccharomyces cerevisiae* and after distillation. The result from this study presents an increase in the fungal count of 3.1×10^2 to 3.5×10^2 CFU/mL from days 1 to 3 and a decrease from 3.5×10^2 to 0 CFU/mL for days 3 to 13 (after recovery by distillation) for the setup fermented by *Aspergillus niger*. There was also an increase in the fungal count of 3.2×10^2 to 3.5×10^2 CFU/mL from days 1 to 3 and a decrease from 3.5×10^2 to 0 CFU/mL for days 3 to 13 for the setup fermented by *Saccharomyces cerevisiae*. Statistically, there was no significant difference between the fungal count for the two setups $p > 0.05$.

Table 1: Change in Fungal Count (CFU/ml) during and after the Production of Bioethanol

Days	<i>Aspergillus niger</i>	<i>Saccharomyces cerevisiae</i>
1	3.1×10^2	3.2×10^2
3	3.5×10^2	3.5×10^2
5	2.6×10^2	2.6×10^2
7	2.0×10^2	2.1×10^2
9	1.2×10^2	1.2×10^2
11	0.1×10^2	0.2×10^2
13	0	0

Figure 1 presents the results of the change in temperature of the setups during fermentation and after distillation (day 13). There was an increase in the temperature from 24.00 °C to 30.35 °C from days 1 to 13 for the setup fermented by *A. niger*. For the set-up fermented by *Saccharomyces cerevisiae*, a temperature increase was also experienced from 25.10 °C to 30.10 °C from days 1 to 13. Statistically, there was no significant difference between the temperatures for both setups, $p = 0.764$.

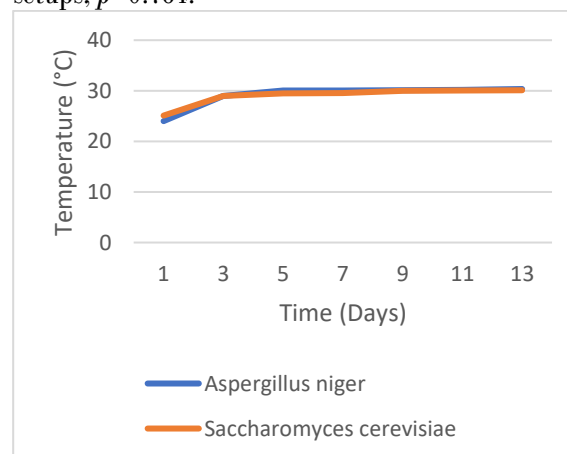


Figure1: Change in Temperature (°C) of the Setups During Fermentation

Figure 2 presents the results of the change in pH during fermentation. A decline in pH was

observed from days 1 to 13, from 5.50 to 3.85 and 5.50 to 4.30 for the setups fermented by *A. niger* and *Saccharomyces cerevisiae*, respectively. Statistically, there was no difference between the pH of the two setups, $p=0.970$.

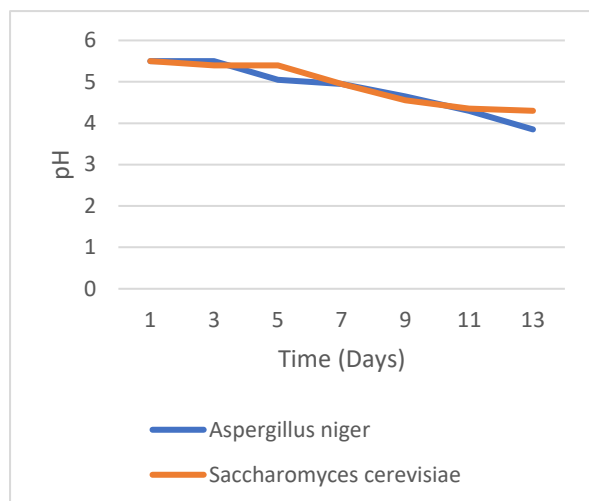


Figure 2: Change in pH of the Setups During Fermentation

Figure 3 presents the result of the change in specific gravity g/ mL of both setups across the days during fermentation and after distillation (day 13). There was a decrease in the specific gravity from 1.018 to 0.436 g/mL from days 1 to 13 for the setup fermented by *A. niger*. For the setup fermented by *Saccharomyces cerevisiae*, a decline in specific gravity was also experienced from 1.012 to 0.322 g/mL from days 1 to 13. Statistically, there was no significant difference between the specific gravity for both setups, $p=0.868$.

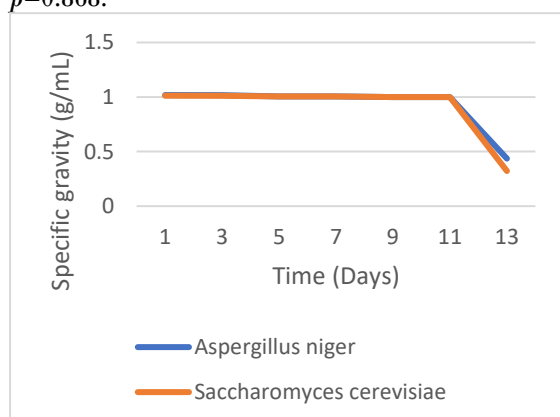


Figure 3: Change in Specific Gravity (g/mL) of the Setups During Fermentation

Figure 4 presents the result of the change in alcohol content (%) in the course of the

fermentation. An increase was observed in the alcohol content from days 1 to 13, from 1.50 to 45.00 % and 1.60 to 50.00 % for the setups fermented by *A. niger* and *Saccharomyces cerevisiae*, respectively. Statistically, there was no difference between the alcohol content of the setups fermented by *A. niger* and *Saccharomyces cerevisiae*, $p=1.00$.

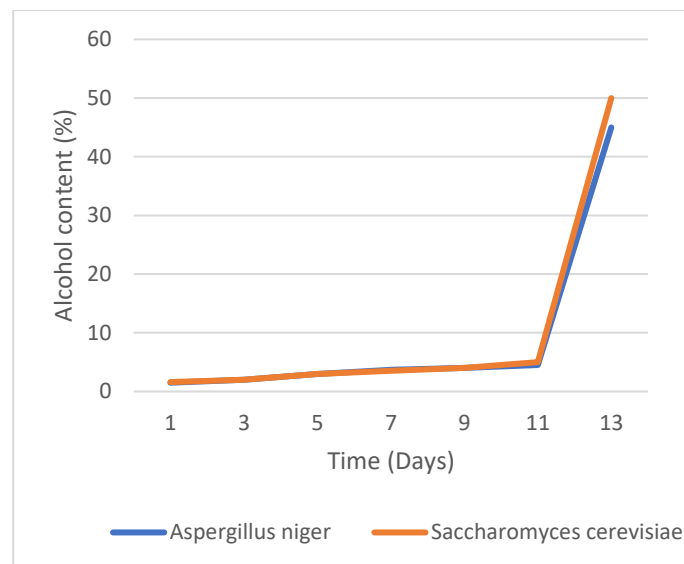


Figure 4: Change in Alcohol Content (%) of the Setups During Fermentation

Discussion

Bioethanol, due to its wide applications and safe use to curb environmental pollution, remains in high demand (Palupi *et al.*, 2020). This study investigated the fungal and physicochemical parameters during the fermentation of bioethanol from plantain (*Musa paradisiaca*) peels.

The fermentation was carried out under aseptic conditions in order to obtain a high-quality fermentation yield. The result obtained from this study revealed an increase in the fungal count from 3.1×10^2 to 3.5×10^2 CFU/mL from days 1 to 3 and a decrease from 3.5×10^2 to 0 CFU/mL from day 3 to 13 (after recovery of the ethanol) for the setup fermented by *Aspergillus niger*. There was also an increase experienced in the count from 3.2×10^2 to 3.5×10^2 CFU/mL from days 1 to 3 and a decrease from 3.5×10^2 to 0 CFU/mL from days 3 to 13 (after recovery of the ethanol) for the setup fermented by *Saccharomyces cerevisiae*. Statistically, there was no significant difference between the counts for the two setups $p>0.05$. A similar study was carried out by Opara & Alabere (2024), and they recorded higher values for fungal count of 4.4×10^2 CFU/mL to 5.9×10^2 for days 1 to 7, and a decrease from 5.9×10^2 CFU/mL to 0 for days 7 to after ethanol distillation for the setup

fermented by *Aspergillus niger*. They also reported higher values for an increase in the fungal count of 1.6×10^2 CFU/mL to 2.9×10^2 for days 1 to 7 and a decrease from 2.9×10^2 CFU/mL to 0 for days 7 to after ethanol distillation for the setup fermented by *Saccharomyces cerevisiae*.

The study revealed an increase in temperature during fermentation, and this could be attributed to the biochemical changes that occurred during the metabolism of the substrates by the fermenting organisms. There was an increase in temperature from 24.00 to 30.35 °C and from 25.10 to 30.10 °C from days 1 to 13 for the setups fermented by *A. niger* and *Saccharomyces cerevisiae*, respectively. The increase in temperature observed in this study is in agreement with the increase in temperature of 25.0 to 30.35 °C reported by Opara and Alabere (2024) in a similar study of bioethanol fermentation from *Musa acuminata* peels.

A decrease in pH was experienced during fermentation. This decrease in pH may be attributed to the accumulation of organic acids produced by the fermenting organisms during fermentation. There was a decrease in the pH from 5.50 to 3.85 from days 1 to 13 for the setup fermented by *A. niger* and from 5.50 to 4.30 from days 1 to 13 for the setup fermented by *Saccharomyces cerevisiae*. The decrease in pH experienced during fermentation agrees with the pH reported by Opara & Alabere (2024), who observed a decline in pH from 5.50 to 4.00 by the fermentative action of *Saccharomyces cerevisiae* and from 5.50 to 3.80 by the fermentative action of *A. niger* for the production of bioethanol from *Musa acuminata* peels. Adedayo *et al.* (2020) carried out a similar study but reported a lower pH from 4.57 to 4.90 during the process of fermentation by *Saccharomyces cerevisiae*. A related study was also conducted by Ajiboye *et al.* (2024), and they reported a decrease in pH from 5.61 to 4.06 during the fermentation of bioethanol by *Saccharomyces cerevisiae*.

A reduction in the specific gravity during the course of the fermentation was observed, and this was due to the conversion of the total solids into ethanol, which is less dense than water. This, in turn, causes a decline in the specific gravity. The decrease in specific gravity could also be attributed to the absorption of water by *Musa paradisiaca* peels, which causes a reduction in the density of the fermenting medium (Timothy *et al.*, 2021). The result of the specific gravity obtained from this study revealed a decrease in the specific gravity from 1.018 to 0.436 g/mL for the setup that was fermented by *A. niger* and from 0.012 to 0.322 g/mL for the setup that was fermented by

Saccharomyces cerevisiae from days 1 to 13 (after recovery of bioethanol by distillation). The result of the specific gravity recorded in this study is in accordance with the findings of Opara and Alabere (2024) who carried out a similar study on bioethanol fermentation from *Musa acuminata* (banana) peels and recorded a decrease in specific gravity from 1.02 to 0.03 g/mL by the fermentative action of *Saccharomyces cerevisiae* and from 1.03 to 0.01 g/mL by the fermentative action of *Aspergillus niger*. A similar study was also carried out by Ajiboye *et al.* (2024), and they reported a decrease in the specific gravity from 0.995 to 0.941 g/mL by the fermentative action of *Saccharomyces cerevisiae*. Adedayo *et al.* (2020) also conducted a similar study on bioethanol fermentation by *Saccharomyces cerevisiae* and reported a decline in the specific gravity from 1.48 to 0.92 g/mL.

A correlation exists between specific gravity and alcohol content. The lower the specific gravity, the higher the alcohol content observed. This could be attributed to the readily available reducing sugar being converted to ethanol during fermentation by *A. niger* and *Saccharomyces cerevisiae*. The study revealed that there was an increase in the alcohol content from 1.50 to 45.00% for the setup that was fermented by *A. niger* and from 1.6 to 50.00% for the setup that was fermented by *Saccharomyces cerevisiae* from days 1 to after the recovery of the bioethanol (day 13). This result is in concurrence with the findings of Opara and Alabere (2024), who carried out a similar study on the fermentation of bioethanol and reported an increase in the alcohol content from 0 to 50.00 % by the fermentative action of *Saccharomyces cerevisiae* and from 0 to 55.0 % by the fermentative action of *Aspergillus niger*. The findings of this study were contradictory to the findings reported by Ajiboye *et al.* (2024), who conducted a similar study and reported a lower alcohol content of 3.48 to 6.93 % by the fermentative action of *Saccharomyces cerevisiae*.

Conclusion

The study aimed to investigate the fungal and physicochemical parameters involved in the fermentation of *Musa paradisiaca* (plantain) peels into bioethanol. Fermentation was carried out in two separate setups, one utilizing *A. niger* and the other *Saccharomyces cerevisiae* as fermenting agents. The fermentation process lasted for 12 days, and on the 13th day, bioethanol was recovered through distillation. The results showed a high alcohol content of 45% and 50% produced by *A. niger* and *Saccharomyces cerevisiae*, respectively. This study highlights that plantain

peels, which are typically considered environmental waste, are effective substrates for bioethanol production, yielding high alcohol content.

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