Prospect on Renewable Energy and Comparative Analysis of Bioethanol Produced from Orange, Banana and Watermelon by Co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae*

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ABSTRACT

Excessive dependence on non-renewable energy sources has contributed to global environmental degradation and rising public health concerns, highlighting the urgent need for sustainable alternatives such as bioethanol. Fruit processing wastes like banana, orange, and watermelon peels are often discarded despite their rich carbohydrate content, making them promising substrates for renewable biofuel production. This study aimed to evaluate the potential of banana, orange, and watermelon peels as low-cost substrates for bioethanol production using locally sourced Aspergillus niger and Saccharomyces cerevisiae, and to compare their ethanol yields under co-culture fermentation. A. niger was isolated from humus-rich soil at the Abubakar Tatari Ali Polytechnic botanical garden, while S. cerevisiae was isolated from the indigenous fermented beverage "Burukutu". Identification was based on cultural, morphological, and biochemical characteristics, followed by screening for enzymatic and fermentative capabilities. Substrates underwent proximate analysis and acid hydrolysis, after which co-culture fermentation (pH 5.5, 8 g substrate, 30°C, 5 days) was conducted. Ethanol levels were monitored across fermentation days. A. niger exhibited strong α-amylase activity with a 47 mm clear zone on starch agar, while S. cerevisiae demonstrated typical budding morphology, thermotolerance up to 40°C, and ethanol tolerance up to 12%. Watermelon peel had the highest carbohydrate content (50.55%), banana peel contained high lipid (24.32%) and fiber (15.30%), and orange peel showed high moisture (42.02%). Ethanol yields peaked on day four at 12.46% (banana), 11.60% (watermelon), and 8.90% (orange), followed by a decline due to substrate depletion and ethanol inhibition. Locally sourced microbial strains and fruit peel wastes are viable resources for bioethanol production, demonstrating strong potential for renewable energy generation and environmentally friendly waste management. This study provides evidence that indigenous microbial strains and readily available fruit wastes can be integrated into sustainable bioethanol production systems. It further establishes the effectiveness of co-culture fermentation and enzymatic hydrolysis in enhancing ethanol yield, supporting circular bioeconomy strategies and low-cost biofuel innovations in developing nations.

Keywords: *Biofuel, Aspergillus niger, Burukutu, Amylase, Sustainable, Fermentation*

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INTRODUCTION

The rising global demand for energy, coupled with the excessive consumption of non-renewable fossil fuels, has intensified environmental degradation and contributed to a range of public health challenges (Kahia et al., 2016; Rahman et al., 2023). These concerns have accelerated the global pursuit of cleaner, renewable, and sustainable

energy alternatives. Among the available options, bioethanol has emerged as a promising renewable fuel due to its biodegradability, low toxicity, and ability to significantly reduce greenhouse gas emissions. Its compatibility with existing transportation systems further enhances its appeal as a feasible substitute for gasoline (Itelima et al., 2012; Khan et al., 2021). Despite its advantages, the commercial viability of bioethanol production remains largely dependent on the cost and availability of feedstock. Consequently, the exploration of inexpensive, abundant, and renewable agricultural wastes as substrates has become a major focus in biofuel research (Franko et al., 2016; Zhang et al., 2020).

Banana peels are one such agro-waste with significant bioconversion potential. With bananas ranking among the most consumed fruits worldwide, banana peels which account for 30–40% of the total fruit weight are generated in large quantities and largely remain unutilized (Emaga et al., 2008; FAO, 2021). Although nutrient-rich and composed of carbohydrates, proteins, fiber, minerals, and natural sugars, banana peels are typically discarded, causing environmental burden (Brooks, 2008; Oyeleke et al., 2020). Their relatively low lignin content, high carbohydrate composition, and rich mineral profile make them an ideal substrate for microbial fermentation and enzymatic hydrolysis in bioethanol production (Hammond et al., 1996; Kumar & Sharma, 2022). These characteristics facilitate the growth of fermenting microbes and enhance the efficiency of starch and cellulose breakdown (Essien et al., 2005; Ogbonna et al., 2021). Similarly, orange peel waste (OPW), a major by-product of citrus juice processing industries, represents another abundant lignocellulosic biomass with significant potential for bioethanol production (Venkata et al., 2013; Haruta et al., 2019). Orange peels contain high levels of cellulose and hemicellulose, which can be hydrolyzed into fermentable sugars following appropriate pretreatment (Kumar et al., 2009; Lin et al., 2021).

Effective pretreatment whether acid, enzymatic, or physicochemical helps disrupt cellulose crystallinity and remove lignin barriers, thereby enhancing sugar accessibility for fermentation (Gautam et al., 2023). Utilizing OPW for bioethanol production not only reduces the environmental load of citrus processing wastes but also supports renewable energy generation, making it a sustainable dual-benefit resource (Mane & Ambekar, 2020). Watermelon (Citrullus lanatus) peel and pulp residues also constitute a promising but underexplored biomass resource. Watermelon is cultivated extensively across tropical and subtropical regions, producing large volumes of organic waste composed of highly fermentable sugars typically 7-10% of its juice content (Kim et al., 1984; FAO, 2016). Its minimal lignin content and high carbohydrate concentration make it an easily degradable substrate suitable for yeast fermentation (Bello et al., 2019; Patel et al., 2022). The valorization of watermelon waste for bioethanol production aligns with global sustainability targets by diverting organic waste from landfills and promoting circular bioeconomy practices (Ezekiel et al., 2021; Shukla et al., 2023). Efficient bioethanol production relies on robust microorganisms capable of withstanding stress conditions such as acidity, heat, ethanol accumulation, and osmotic pressure (Sean & Johann, 2015; Raghuwanshi & Kuila, 2020). *Saccharomyces cerevisiae* remains the most widely used fermenting organism owing to its high substrate conversion rate, ethanol tolerance, and metabolic versatility (Khan et al., 2021; Adesanya et al., 2022).

Recent advancements in microbial biotechnology have improved the performance of *S. cerevisiae*, particularly its ability to ferment multiple sugar types derived from lignocellulosic hydrolysates, (Patel et al., 2022; Hu et al., 2023). On the other hand, filamentous fungi such as *Aspergillus niger* are highly valued for their strong enzymatic activities including amylases, cellulases, and pectinases which enhance the hydrolysis of complex plant polymers into fermentable sugars. The synergy between *A. niger* and *S. cerevisiae* in co-culture systems has shown great promise in enhancing bioethanol yield due to simultaneous hydrolysis and fermentation. Together, these factors highlight the potential of fruit peel wastes banana, orange, and watermelon as cost-effective and environmentally sustainable substrates for bioethanol production using a co-culture of *A. niger* and *S. cerevisiae*. This study therefore explores their comparative suitability, contributing to ongoing efforts geared toward renewable energy development and organic waste valorization.

MATERIALS AND METHODS

Isolation and characterization of *Aspergillus niger*

Sample collection and fungal isolation

Aspergillus niger was isolated from humus-rich soil collected from the Abubakar Tatari Ali Polytechnic (ATAP) botanical garden. Soil samples were collected randomly from the top 2 cm layer at four points (≈30 g per point) into sterile polythene bags and transported to the laboratory. Samples were air-dried for 48 hours and sieved to remove debris. One gram of soil was suspended in 9 ml sterile distilled water supplemented with streptomycin (0.005 g) to inhibit bacterial growth. Serial dilutions were prepared, and 0.1 ml aliquots from the 10^{-5} dilution were plated onto Sabouraud Dextrose Agar (SDA). Plates were incubated at 28°C for 5 days. Colonies with typical *A. niger* morphology were purified by repeated sub-culturing (Kumar et al., 2019; Sharma & Singh, 2021).

Cultural Examination

Colony characteristics color, texture, sporulation pattern were examined on SDA and compared to recent fungal identification guidelines (Houbraken et al., 2020; Visagie et al., 2017).

Microscopic examination

A small portion of fungal mycelium was mounted in lactophenol cotton blue and examined under 10× and 40× objectives. Features such as conidial heads, septate hyphae, and vesicle morphology were compared with

modern mycological atlases (Pitt & Hocking, 2022; Samson et al., 2019).

Screening for Alpha-Amylase Production

Amylase activity was assessed using the starch hydrolysis assay. Fungal isolates were inoculated onto starch agar and incubated at 28°C for 72 hours. Plates were flooded with iodine solution; clear zones indicated starch hydrolysis. The isolate with the largest zone of clearance was selected for fermentation (Singh et al., 2021; Sharma et al., 2020).

Isolation and characterization of *saccharomyces cerevisiae*

Yeast isolation

Saccharomyces cerevisiae was isolated from the local fermented beverage "burukutu" obtained in Bauchi. A 0.1 ml aliquot of a 10^{-5} dilution was plated on Malt Yeast Peptone Glucose (MYPG) agar and incubated at 30°C for 48 hours. Colonies resembling *S. cerevisiae* were purified by sub-culturing (Bello et al., 2019; Ayobami et al., 2021).

Microscopic identification

Lactophenol cotton blue wet mounts were prepared and examined under an oil-immersion objective (100×). Oval-shaped cells with multilateral budding confirmed *S. cerevisiae* identity (Sakandar et al., 2019; Ayobami et al., 2021).

Thermotolerance test

Purified yeast isolates were streaked on SDA plates and incubated at 25–45°C for 48 hours. Growth intensity was recorded (Adesanya et al., 2022; Narisetty & Sravanthi, 2020).

Ethanol tolerance test

Yeast cells were inoculated into yeast extract broth containing 3–18% (v/v) ethanol and incubated at 30°C for 48 hours. Growth was measured at OD_{600} (Khan et al., 2021; Narisetty & Sravanthi, 2020).

Glucose tolerance (10–50%)

Isolates were grown in basal medium containing 10–50% glucose at 30°C for 48 hours, and growth was read at OD_{600} (Ibrahim et al., 2018; Khan et al., 2021).

Sugar fermentation test

Basal medium supplemented with individual sugars (glucose, sucrose, fructose, maltose, galactose, lactose) and bromothymol blue indicator was dispensed into tubes containing Durham tubes. Tubes were inoculated and incubated at 30°C for 72 hours. Gas accumulation indicated fermentation (Oyeleke et al., 2020; Ayobami et al., 2021).

Preparation of orange, banana, and watermelon peel substrates

Fruit peels were collected from Wunti Market, Bauchi. Peels were washed, cut into 2 cm pieces, and sun-dried for 14 days. Dried samples were milled into powder and stored in airtight containers until use (Bello et al., 2019; Falade et al., 2021).

Proximate analysis

Proximate composition (moisture, ash, lipid, protein, fiber, carbohydrate) was determined using AOAC (2016) standard procedures.

Pretreatment of substrates

Steam explosion

Ten grams of each fruit peel powder were suspended in 100 ml distilled water and autoclaved at 121°C for 1 hour to achieve steam explosion (Gautam et al., 2023; Sharma & Singh, 2021).

Acid hydrolysis

Dilute sulfuric acid hydrolysis was carried out following the modified protocol of recent lignocellulose hydrolysis studies. Hydrolyzed samples were filtered and the filtrate was used as the fermentation substrate (Gautam et al., 2023; Mankar et al., 2021).

Fermentation process

Fermentation medium

The fermentation medium contained soluble starch, glucose, peptone, malt extract, yeast extract, $MgCl_2$, $CaCO_3$, $(NH_4)_2HPO_4$, and $FeSO_4$. pH was adjusted to 6.0 before sterilization (Adesanya et al., 2022; Sharma et al., 2020).

Inoculum preparation

Both microorganisms were grown in 200 ml Erlenmeyer flasks at 30°C and 200 rpm for 48–72 hours (Byarugaba et al., 2019; Adesanya et al., 2022).

***A. niger* spore suspension**

Spores from 5-day SDA plates were harvested in sterile water with 2.5% Tween 80. Spore concentration was standardized to 2.5×10^5 spores/ml using a haemocytometer (Sharma & Singh, 2021).

***S. cerevisiae* suspension**

Yeast cells grown in Yeast Peptone Dextrose broth were

centrifuged, washed, and standardized to McFarland 1 (~3 × 10^6 cells/ml) (Ayobami et al., 2021; Narisetty & Sravanthi, 2020).

Fermentation setup

Fermentation was performed in 500 ml flasks containing 4% (w/v) peel substrate in 200 ml medium. Flasks were sterilized, inoculated with both microorganisms, and incubated at 30°C with shaking at 200 rpm for 5 days (Adesanya et al., 2022).

Determination of ethanol content

Every 24 hours, 30 ml aliquots were distilled, and ethanol concentration was estimated from specific gravity readings using a standard calibration curve (Zhang et al., 2020; Oyeleke et al., 2020).

Distillation

Distillation was carried out at 78°C, and condensate was collected in a sterile flask (Zhang et al., 2020).

RESULTS AND DISCUSSION

Isolation and characterization of *Aspergillus niger*

Macroscopic and Microscopic Characteristics

A total of four fungal isolates (ATG A-D) were obtained from humus-rich soil samples. Macroscopic characterization revealed variation in colony color, growth rate, and elevation, as shown in (Table 1). Notably, isolate ATG C exhibited the fastest growth, forming green-to-dark green colonies. Microscopic analysis revealed that all isolates possessed branched septate hyphae, with conidiophores ranging from globose to dome-shaped and conidial pigmentation varying among isolates (Table 2). The observed morphological diversity indicates metabolically versatile strains capable of adapting to various fermentation conditions. The rapid growth and characteristic morphology of ATG C suggest its potential for efficient enzymatic hydrolysis of polysaccharides, an important factor in enhancing bioethanol yields (Samson et al., 2019; Pitt & Hocking, 2022).

α-Amylase Production

α-Amylase activity varied among isolates, with ATG C exhibiting the highest activity (47 mm zone of clearance) (Table 3). High α-amylase activity ensures effective starch hydrolysis into fermentable sugars, enhancing ethanol production during co-culture fermentation with *Saccharomyces cerevisiae* (Singh et al., 2021). The high enzymatic activity observed in ATG C aligns with reports by Samson et al. (2019) and Pitt & Hocking (2022), which note that rapidly growing green *A. niger* strains generally exhibit strong starch-degrading capacity. These results underscore the importance of selecting high enzyme-producing strains for bioethanol production from starch-rich substrates.

Isolation and characterization of *saccharomyces cerevisiae*

Macroscopic traits and sugar fermentation

Yeast isolates (BKa-BKd) formed creamy to whitish smooth colonies (Table 4). All isolates fermented glucose, sucrose, and fructose, while maltose and galactose were partially fermented; lactose was not fermented. Isolates BKc and BKd showed the broadest sugar utilization spectrum. The ability to metabolize multiple sugars is essential for bioethanol production from complex fruit peel substrates containing mono- and disaccharides. The non-fermentation of lactose is consistent with the metabolic profile of *S. cerevisiae* (Oyeleke et al., 2020).

Glucose, ethanol, and thermotolerance

Yeast growth decreased with increasing glucose concentration, with optimal growth at 10% and minimal growth at 50% (Table 5). Ethanol tolerance was observed up to 12% (OD_{600} = 1.04 ± 0.001), with higher concentrations inhibiting growth (Table 6). Thermotolerance testing revealed optimal growth at 30°C (+++), moderate growth at 25-35°C (++), minimal growth at 40°C (+), and no growth at 45°C (Table 7). These results are in agreement with Adekunle et al. (2018) and Oyeleke et al. (2020), who reported that industrially viable *S. cerevisiae* strains exhibit optimal growth under moderate sugar and ethanol concentrations. The mesophilic temperature preference aligns with observations in tropical bioethanol studies (Bello et al., 2019), confirming the suitability of these isolates for fermentation in ambient conditions.

Proximate composition of fruit peel substrates

The proximate composition of banana, orange, and watermelon peels is summarized in (Table 8). Banana peel was rich in lipids (24.32%) and fiber (15.30%), watermelon contained the highest carbohydrate content (50.55%), and orange peel exhibited the highest moisture content (42.02%). High carbohydrate content in watermelon facilitates rapid fermentation, while fiber and lipid-rich banana peel supports sustained enzymatic activity. Orange peel's high moisture may dilute fermentable sugars, suggesting the need for optimized pretreatment methods such as concentration or acid hydrolysis (Bello et al., 2019). The results are consistent with Bello et al. (2019), who reported watermelon peels as ideal substrates for sugar-based fermentation and highlighted banana peel's role in sustaining enzymatic activity. Rahman et al. (2023) also emphasized pretreatment optimization for high-moisture substrates such as orange peel.

Ethanol production from fruit peel wastes

Ethanol yields increased progressively, reaching a peak

Table 1: Macroscopic Characteristics of *A. niger* Isolates.

Isolate	Surface Colour	Margin	Reverse Side	Elevation	Growth
ATG A	Pinkish	Entire	Pale to bright yellow	Umbonate	Moderate
ATG B	Dark brown/Black	Entire	Colourless	Umbonate	Rapid
ATG C	Green/Dark Green	Entire	Colourless/yellow	Umbonate	Rapid
ATG D	Yellow-Greyish Green	Entire	Colourless/yellow	Umbonate	Moderate

Table 2: Microscopic Characteristics of *A. niger* Isolates.

Isolate	Hyphae	Conidiophore	Conidia
ATG A	Branched septate	Globose	Creamish
ATG B	Branched septate	Globose	Blackish brown
ATG C	Branched septate	Dome-shaped	Blue green
ATG D	Branched septate	Globose	Yellowish green

Table 3: α-Amylase Activity of *A. niger* Isolates (mm).

Isolate	Zone of Clearance
ATG A	26
ATG B	35
ATG C	47
ATG D	22

Table 4: *S. cerevisiae* Macroscopic Characteristics and Sugar Fermentation.

Isolate	Colony Colour	Colony Nature	Elevation	Appearance	Margin	Sucrose	Maltose	Lactose	Fructose	Galactose	Glucose
BKa	Whitish	Smooth/Shining	Concave	Ovoid	Entire	+	-	-	+	+	+
BKb	Whitish cream	Smooth	Raised	Yeast-like	Wavy	+	-	-	+	-	+
BKc	Cream	Smooth	Concave	Yeast-like	Entire	+	+	-	+	+	+
BKd	Cream	Smooth	Convex	Glabrous	Entire	+	+	-	+	-	+

Table 5: Growth of *S. cerevisiae* in Glucose (10–50%) After 48h.

Glucose (%)	OD_{600} ± SD
10	3.064 ± 0.002
20	2.007 ± 0.019
30	1.325 ± 0.017
40	0.903 ± 0.025
50	0.786 ± 0.001

Table 6: Ethanol Tolerance of *S. cerevisiae* (OD_{600} ± SD).

Ethanol (%)	OD_{600} ± SD
3	2.34 ± 0.008
6	1.72 ± 0.012
9	1.43 ± 0.016
12	1.04 ± 0.001
15	0.87 ± 0.003
18	0.34 ± 0.002

Table 7: Thermotolerance of *S. cerevisiae.*

Temperature (°C)	Growth Level
25	++
30	+++
35	++
40	+
45	–

on day 4: banana (12.46%), watermelon (11.60%), and orange (8.90%) (Table 9). A decline in yield was observed on day 5, likely due to substrate depletion and ethanol inhibition. Banana peel's superior yield demonstrates that substrates with high fiber and lipid content can sustain fermentation and enzymatic activity for longer periods. Watermelon's carbohydrate-rich composition supports rapid sugar conversion, while orange peel's lower yield indicates that pretreatment methods may need optimization. These findings are consistent with Singh et

Table 8: Proximate Composition of Fruit Peels (%)

Parameter	Banana	Orange	Watermelon
Moisture	12.62	42.02	32.46
Ash	10.10	3.80	6.87
Fiber	15.30	7.61	5.20
Protein	4.92	4.22	3.80
Lipid	24.32	3.60	1.12
Carbohydrate	32.74	38.75	50.55

Table 9: Ethanol Yield (%) from Fruit Peel Wastes (pH 5.5, 8g Substrate, 30°C).

Day	Watermelon	Orange	Banana
1	1.68	1.72	1.78
2	4.93	6.32	6.52
3	7.88	6.92	8.64
4	11.60	8.90	12.46
5	10.02	7.42	10.20

al. (2021) and Bello et al. (2019), who reported that co-culture fermentation of *A. niger* and *S. cerevisiae* effectively converts agro-wastes into ethanol. The slight decline in ethanol yield after peak fermentation is similar to observations in other studies, reflecting substrate exhaustion and ethanol inhibition (Adekunle et al., 2018). This study demonstrates that local fruit peel wastes can serve as cost-effective substrates for bioethanol production. *A. niger* ATG C and *S. cerevisiae* BKc/BKd were identified as promising microbial strains for co-culture fermentation. The findings provide a foundation for scaling up renewable energy production using locally sourced agro-wastes, supporting sustainable bioenergy initiatives and circular bioeconomy practices in developing regions.

Conclusion

This study shows that Aspergillus niger and Saccharomyces cerevisiae, naturally found in the environment, have strong enzymatic and fermentation capabilities that make them excellent candidates for bioethanol production. Using fruit wastes such as banana, orange, and watermelon peels not only offers a greener alternative to fossil fuels but also helps manage agricultural waste and supports local economies (Samantaray & Mohapatra, 2023; Yasin, 2025). While challenges like high lignin content and production costs still exist, recent improvements in pretreatment methods, microbial strain development, and co-fermentation techniques are helping to boost efficiency (Bušić, Skoko, Koprivanac, & Ng, 2018; Ajayi et al., 2023). Moving forward, efforts should focus on fine-tuning the conditions for co-culturing these microbes and combining enzymatic breakdown with fermentation processes, ensuring a sustainable and cost-effective approach to turning fruit wastes into bioethanol.

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Conflict of interests

The authors of this work declared no conflicting interest.

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