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Assessment of Different Agricultural Waste Feedstock Options in Makurdi for Bioethanol Production

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Abstract

The growing demand for sustainable and environmentally friendly energy sources has intensified interest in bioethanol production from renewable biomass, particularly lignocellulosic agricultural residues. This study evaluated the bioethanol production potential of selected agro-wastes including corn cob, rice straw, cassava peels, sawdust, and groundnut shells, sourced from Makurdi, Benue State, Nigeria, with the aim of identifying a feasible and sustainable feedstock for renewable fuel generation. The substrates were subjected to alkaline pre-treatment using sodium hydroxide to enhance saccharification, followed by fermentation using *Saccharomyces cerevisiae* (PWA) under controlled laboratory conditions. Variations in pH, reducing sugar concentration, ethanol yield, and yeast growth dynamics were monitored over a four-day fermentation period. The results revealed substrate-dependent fermentation behavior. pH values remained predominantly within a slightly alkaline range across all substrates, with cassava peels exhibiting a progressive decline from pH 8.0 to 6.0, while groundnut shells showed an increase to pH 9.1 by day four. Reducing sugar concentrations decreased over time in all substrates, indicating active microbial utilization, with rice straw recording the highest initial reducing sugar concentration (0.076 ± 0.001 g/g). Ethanol production peaked between days one and two, depending on the substrate, with cassava peels yielding the highest ethanol concentration (0.045 ± 0.001 g/g). Yeast growth trends correlated positively with fermentable sugar availability, as cassava peels consistently supported the highest *Saccharomyces cerevisiae* (PWA) population, reaching 4.82×10^6 CFU/mL by day four. Overall, cassava peels demonstrated superior fermentability, ethanol productivity, and micro-

bial support compared to the other agricultural residues evaluated. The findings highlight the significant potential of locally available lignocellulosic wastes for sustainable bioethanol production and underscore their role in waste valorization, greenhouse gas mitigation, and renewable energy development. This study provides valuable insights for advancing biofuel technologies in Nigeria and contributes to efforts toward achieving the United Nations Sustainable Development Goal 7 on affordable and clean energy.

Keywords

Bioethanol Production, Lignocellulosic Biomass, Agricultural Residues, Alkaline Pretreatment, *Saccharomyces cerevisiae* (PWA) Fermentation

1. Introduction

The growing global demand for energy, coupled with the environmental consequences of fossil fuel consumption, has intensified the search for sustainable, renewable, and environmentally friendly energy sources. In response to these challenges, the United Nations adopted the Sustainable Development Goals (SDGs) in 2015, with Goal 7 emphasizing the need to ensure access to affordable, reliable, and sustainable modern energy for all [1]. Biofuels, particularly bioethanol, fall within the core mandate of SDG 7 due to their renewable nature, lower greenhouse gas emissions, and potential to reduce dependence on fossil-derived fuels.

Bioethanol is a renewable biofuel produced through the fermentation of biomass-derived sugars and is widely recognized as a viable substitute or blending component for conventional gasoline. Globally, bioethanol production has increased significantly, reaching approximately 110 billion liters in 2018 and projected to exceed 140 billion liters by 2022, dominated by major producers such as the United States, Brazil, the European Union, and Canada [2]. Despite this global growth, Nigeria remains largely dependent on imports to meet its domestic bioethanol demand.

Nigeria's energy sector continues to face persistent challenges, including inadequate electricity supply, rising fuel costs, and heavy reliance on fossil fuels. Recent estimates indicate that less than 40% - 50% of the Nigerian population has access to reliable electricity, placing the country among those with the highest energy access deficits globally [3]. Although Nigeria is a major crude oil producer, fluctuations in petroleum prices, supply instability, and environmental degradation associated with fossil fuel exploration and utilization have undermined energy sustainability. Fossil fuel combustion contributes significantly to greenhouse gas emissions, air pollution, climate change, and ecosystem damage, while oil spillages have rendered vast areas of land and aquatic ecosystems unproductive.

In addition to energy insecurity, the indiscriminate disposal of agricultural residues poses serious environmental and public health concerns in Nigeria. Agricultural wastes such as corn cobs, rice straw, cassava peels, sawdust, and ground-

nut shells are often left to decay or openly burned, leading to air pollution, greenhouse gas emissions, blockage of drainage systems, and the proliferation of disease vectors. Paradoxically, these lignocellulosic residues represent abundant, low-cost, and renewable feedstocks that can be converted into bioethanol, thereby transforming waste into wealth while simultaneously addressing energy and environmental challenges.

Nigeria reportedly produces approximately 134 million liters of ethanol annually, which accounts for only about 3% of its estimated national demand of over 5.14 billion liters per year, resulting in a substantial demand-supply gap exceeding 5 billion liters [4]. This shortfall highlights the urgent need for local, sustainable bioethanol production pathways that utilize non-food biomass resources. Unlike first-generation bioethanol feedstocks that compete directly with food supplies, lignocellulosic agricultural wastes offer a sustainable alternative and help mitigate the food-fuel conflict.

Lignocellulosic biomass is primarily composed of cellulose, hemicellulose, and lignin, with cellulose and hemicellulose serving as sources of fermentable sugars following appropriate pre-treatment and hydrolysis. Cellulose-rich agricultural residues contain six-carbon sugars that are essential for efficient bioethanol fermentation [5]. However, the complex structure of lignocellulosic materials necessitates pre-treatment to disrupt lignin and enhance enzymatic accessibility to polysaccharides. Among the various pre-treatment strategies, alkaline pre-treatment using sodium hydroxide (NaOH) has been shown to be effective in lignin removal and improvement of sugar yield [6].

Bioethanol production from agricultural residues typically involves several key stages: collection and preparation of biomass, pre-treatment, saccharification to release fermentable sugars, microbial fermentation, and downstream recovery of ethanol through distillation. The economic feasibility of bioethanol production is strongly influenced by feedstock and substrate costs, which may account for up to 80% of total production expenses when refined sugars such as glucose are used [7]. Consequently, the utilization of inexpensive and locally available lignocellulosic wastes significantly enhances the sustainability and economic viability of bioethanol production systems.

Although alternative feedstocks such as microalgae have been explored for biofuel production due to their high growth rates and carbon fixation potential, large-scale application remains constrained by low biomass productivity and costly downstream processing [8]. While studies conducted in Nigeria have demonstrated the potential of microalgae-based biofuel systems, particularly when integrated with wastewater treatment [9] [10], lignocellulosic agricultural wastes remain more practical due to their abundance, low cost, and ease of sourcing, especially in agrarian regions such as Benue State.

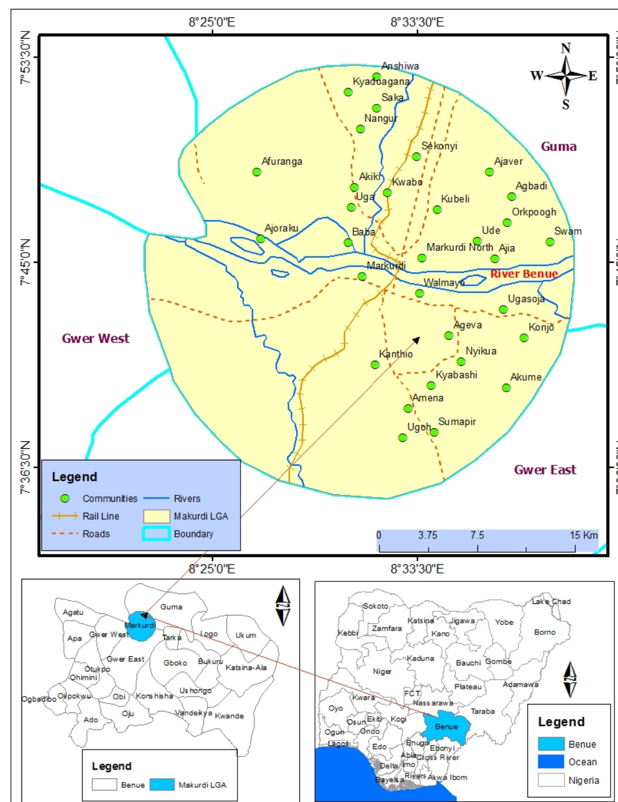
Benue State, often referred to as the "food basket" of Nigeria, generates large volumes of agricultural residues annually. These wastes are continuously released into the environment, creating disposal challenges while remaining largely un-

derutilized for energy generation. Harnessing these residues for bioethanol production offers multiple benefits, including rural economic empowerment, job creation, waste reduction, and mitigation of environmental pollution. Furthermore, bioethanol production from agricultural wastes aligns with global circular economy principles and international directives promoting waste reduction, recycling, and sustainable resource management [11].

Based on these considerations, the present study was designed to evaluate the suitability of selected lignocellulosic agricultural wastes—namely corn cobs, rice straw, cassava peels, sawdust, and groundnut shells—for bioethanol production. Specifically, the study determined and compared the ethanol yield obtained from the fermentation of these pre-treated substrates. The conceptual framework of the study was adapted from established lignocellulosic bioethanol production models involving biomass pre-treatment, hydrolysis, yeast fermentation, and ethanol recovery [12]. The findings of this study are expected to contribute to the development of sustainable bioethanol production strategies and support Nigeria's efforts toward achieving SDG 7 through the utilization of locally available agricultural wastes.

2. Materials and Methods

2.1. Study Area



(Source: Ministry of Lands and Survey, Makurdi, 2011).

Figure 1. Map of the study area (Joseph Sarwuan Tarka University, Makurdi).

The study was conducted in Makurdi, the capital city of Benue State, Nigeria. Makurdi lies within the Benue Trough along the River Benue floodplain and is geographically located at approximately latitude 7˚44' N and longitude 8˚32' E, with an average elevation of 113 m above sea level. The area falls within the Southern Guinea Savannah ecological zone (**Figure 1**) and is characterized by a tropical climate with distinct wet and dry seasons. Agriculture constitutes the predominant economic activity in the region, with extensive cultivation of cassava, rice, maize, groundnut, yam, and other crops, resulting in the generation of large volumes of agricultural residues.

Laboratory experiments were carried out at the Department of Microbiology, Joseph Sarwuan Tarka University, Makurdi (JOSTUM), located in the North Bank area of the city. The institution provides suitable facilities for microbiological and biotechnological research.

2.2. Sample Collection and Preparation

Agricultural residues used in this study included corn cobs, rice straw, cassava peels, sawdust, and groundnut shells. The materials were sourced from local farms, sawmills, and processing sites within Makurdi. All samples were thoroughly washed with tap water followed by distilled water to remove adhering soil and debris.

The cleaned samples were sun-dried for five to seven days and further oven-dried at 60˚C for 24 hours to reduce moisture content. The dried materials were reduced in size using a hammer and mortar and subsequently milled using a commercial milling machine. The powdered substrates were sieved to obtain uniform particle sizes of approximately 1 - 2 mm and stored in clean polyethylene bags at room temperature until further use.

2.3. Laboratory Equipment and Reagents

2.3.1. Laboratory Equipment

The equipment used included sterile beakers, conical flasks, measuring cylinders, glass funnels, spatulas, and Petri dishes for sample handling and preparation. An Ohaus analytical balance was used for accurate weighing of samples and reagents. Absorbance measurements were obtained using a Jenway UV-Visible spectrophotometer. A thermostatically controlled water bath (Model HH-6), dry hot-air oven (Model DHG-9053A), orbital shaker incubator, and autoclave were employed for heating, incubation, and sterilization processes. pH measurements were conducted using a Hanna digital pH meter. Whatman No. 1 filter paper was used for filtration.

2.3.2. Chemicals and Culture Media

Analytical-grade reagents were used throughout the study. These included sodium hydroxide (NaOH), dinitrosalicylic acid (DNS) reagent, magnesium sulphate ($MgSO_4$), dipotassium hydrogen phosphate (K_2HPO_4), ammonium sulphate $(NH_4)_2SO_4$), peptone, yeast extract, potassium dichromate ($K_2Cr_2O_7$), sul-

phuric acid (H_2SO_4), and absolute ethanol. Potato Dextrose Agar (PDA) and agar-agar were used for yeast cultivation.

2.4. Sterilization Procedure

All glassware was washed with detergent, rinsed with distilled water, air-dried, and sterilized in a hot-air oven at 160˚C for one hour. Culture media and distilled water were sterilized by autoclaving at 121˚C for 15 minutes. Work surfaces were disinfected with 95% ethanol before and after laboratory procedures to maintain aseptic conditions.

2.5. Preparation of Culture Media

Potato Dextrose Agar (PDA) was prepared following the manufacturer's instructions. Briefly, 39 g of dehydrated PDA powder was dissolved in 1 L of distilled water with gentle agitation. The medium was sterilized at 121˚C for 15 minutes and allowed to cool to approximately 45˚C before being aseptically dispensed into sterile Petri dishes and left to solidify.

2.6. Alkaline Pre-Treatment of Lignocellulosic Substrates

Alkaline pre-treatment was employed to enhance the digestibility of the lignocellulosic substrates by disrupting lignin and hemicellulose structures, following modified procedures described by Afzal *et al.* [5].

Fifty grams (50 g) of each powdered substrate was soaked in 500 mL of 2% (w/v) sodium hydroxide solution and incubated at room temperature for two hours. The mixtures were subsequently heated at 100˚C for I hour in a water bath (Model: HH-6). After treatment, the slurry was allowed to cool to room temperature and filtered using Whatman No. 1 filter paper. This was washed repeatedly with distilled water until a neutral pH was attained. The pre-treated samples were oven-dried at 60˚C for 24 hours and stored for fermentation experiments.

2.7. Alcoholic Fermentation

Three grams (3 g) of NaOH hydrolyzed substrate was dispensed into 1 L conical flasks containing fermentation nutrients comprising 0.2% $MgSO_4$, 0.3% K_2HPO_4, 0.3% $(NH_4)_2SO_4$, 0.3% peptone, and 0.3% yeast extract. Sterile distilled water was added to achieve the desired volume. The medium was sterilized by autoclaving at 121 for 15 mins. Thereafter, it was inoculated aseptically with *Saccharomyces cerevisiae* (PWA) cells isolated from palm wine which was subsequently harvested from PDA plates and suspended in sterile saline solution. Fermentation proceeded at 30˚C for 96 hours in an orbital shaker incubator of agitation speed of 40 rpm. Samples were withdrawn at 24-hour intervals for the determination of reducing sugars, ethanol concentration, and pH.

2.8. Determination of Reducing Sugar Concentration

Reducing sugars were quantified using the DNS method as described by Miller

[13], with slight modifications. Fermentation samples were centrifuged at 5000 rpm for 10 minutes. One milliliter of the supernatant was mixed with 1 mL of DNS reagent and heated in a boiling water bath for five minutes. After cooling, the mixture was diluted to 50 mL with distilled water, and absorbance was measured at 540 nm.

A glucose standard curve (0.2 - 1.0 g/g) was prepared, and reducing sugar concentrations were determined by interpolation from the calibration curve [13] [14].

2.9. Determination of Ethanol Concentration

Ethanol concentration was determined using the acidic potassium dichromate method described by Caputi, Ueda and Brown [15]. One milliliter of centrifuged fermentation broth was mixed with 5 mL of potassium dichromate reagent and incubated at 60˚C for 20 minutes. Absorbance was measured at 578 nm using a UV-Visible spectrophotometer.

Ethanol standards (0% - 10% v/v) were prepared and treated similarly to generate a calibration curve, from which ethanol concentrations in the samples were extrapolated [15].

2.10. Yeast Enumeration

Enumeration of yeast load during fermentation was monitored using serial dilution and pour plate techniques. Aliquots (0.1 mL) of appropriately diluted fermentation broth were plated on PDA and incubated at 30˚C for 48 hours, after which colonies were counted.

2.11. pH Measurement

The pH of the fermenting broth was measured at 24-hour intervals using a calibrated Hanna digital pH meter by directly immersing the electrode into the samples.

2.12. Data Analysis

All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation. Data were analyzed using appropriate statistical tools to ensure reliability and reproducibility of the findings.

3. Results

3.1. Variation in pH during Fermentation of Agricultural Residues

Changes in pH during the fermentation of the different lignocellulosic substrates are presented in **Table 1**. Across all substrates, the pH values remained within a slightly alkaline range throughout the fermentation period. On day 1, pH values ranged from 7.9 ± 0.03 (rice straw) to 8.2 ± 0.02 (groundnut shells). Fluctuations were observed as fermentation progressed, with cassava peels exhibiting a progressive decrease in pH from 8.0 ± 0.03 on day 1 to 6.0 ± 0.01 by day 4. In contrast,

groundnut shells showed an increasing trend, reaching the highest pH value of 9.1 ± 0.30 on day 4. Corn cob, rice straw, and sawdust showed relatively stable pH values with minor variations over the four-day fermentation period.

Table 1. Variations in pH during fermentation of substrates.

Day	Corn cob	Rice straw	Cassava peels	Sawdust	Groundnut shells
1	8.1 ± 0.00	7.9 ± 0.03	8.0 ± 0.03	8.0 ± 0.09	8.2 ± 0.02
2	8.5 ± 0.06	8.4 ± 0.01	7.5 ± 0.03	8.1 ± 0.06	8.7 ± 0.10
3	7.8 ± 0.05	8.0 ± 0.06	7.0± 0.07	7.73 ± 0.20	8.8 ± 0.20
4	8.0 ± 0.00	8.3 ±0.05	6.0 ± 0.01	8.0 ± 0.05	9.1 ± 0.30

a. Values are mean ± standard deviation.

3.2. Reducing Sugar Concentration during Fermentation (g/g)

The concentration of reducing sugars during fermentation is shown in Table 2. On day 1, rice straw recorded the highest reducing sugar concentration (0.076 ± 0.001 g/g), while corn cob exhibited the lowest value (0.010 ± 0.004 g/g). As fermentation progressed, reducing sugar concentrations generally declined across all substrates, although fluctuations were observed depending on the substrate type. By day 4, reducing sugar concentrations ranged from 0.002 ± 0.013 g/g in corn cob to 0.020 ± 0.004 g/g in cassava peels.

Table 2. Reducing sugar concentration during fermentation (g/g).

Day	Corn cob	Rice straw	Cassava peels	Sawdust	Groundnut shells
1	0.010 ± 0.004	0.076 ± 0.001	0.027 ± 0.002	0.018 ± 0.001	0.0143 ± 0.004
2	0.014 ± 0.007	0.026 ± 0.004	0.027 ± 0.004	0.015 ± 0.003	0.0183 ± 0.002
3	0.001 ± 0.010	0.018 ±0.002	0.018 ± 0.001	0.0193 ± 0.002	0.013 ± 0.001
4	0.002 ± 0.013	0.008 ±0.001	0.020 ± 0.004	0.014 ± 0.003	0.0153 ± 0.003

a. Values are mean ± standard deviation, reducing sugar yield (g/g), the unit denotes grams of reducing sugars (expressed as glucose equivalents) produced per gram of dry lignocellulosic biomass used in the fermentation.

3.3. Ethanol Concentration during Fermentation (g/g)

Ethanol production during the fermentation of the agricultural residues is presented in Table 3. On day 1, cassava peels produced the highest ethanol concentration (0.045 ± 0.001 g/g), while rice straw produced the lowest (0.006 ± 0.001 g/g). Peak ethanol concentrations were generally observed between days 1 and 2, depending on the substrate. On day 2, groundnut shells (0.0383 ± 0.001 g/g) and sawdust (0.037 ± 0.001 g/g) recorded their highest ethanol levels. A decline in ethanol concentration was observed across all substrates by day 4.

Table 3. Ethanol concentration during fermentation of agricultural residues (g/g).

Day	Corn cob	Rice straw	Cassava peels	Sawdust	Groundnut shells
1	0.032 ± 0.001	0.006 ± 0.001	0.045 ± 0.001	0.013 ± 0.001	0.0260 ± 0.005
2	0.015 ± 0.003	0.018 ± 0.001	0.038 ± 0.002	0.037 ± 0.001	0.0383 ± 0.001
3	0.012 ± 0.005	0.006 ± 0.001	0.017 ± 0.001	0.0183 ± 0.003	0.0143 ± 0.002
4	0.010 ± 0.004	0.005 ± 0.001	0.013 ± 0.002	0.0103 ± 0.005	0.0113 ± 0.002

a. Values are mean ± standard deviation, Ethanol yield (g/g), the unit refers to grams of ethanol produced per gram of dry substrate initially loaded into the fermentation system.

3.4. Growth Profile of *Saccharomyces* Species during Fermentation

The growth dynamics of *Saccharomyces* species (PWA) during fermentation are shown in Table 4. Yeast populations increased across all substrates from day 1 to day 3, indicating active microbial growth during fermentation. Cassava peels consistently supported the highest yeast counts, reaching a maximum of 4.82×10^6 CFU/mL on day 4. Corn cob and rice straw showed moderate growth patterns, while sawdust supported comparatively lower yeast densities throughout the fermentation period. A slight decline or stabilization in cell counts was observed by day 4 in some substrates.

Table 4. Time course of *Saccharomyces* species PWA growth during fermentation (CFU/mL).

Day	Corn cob	Rice straw	Cassava peels	Sawdust	Groundnut shells
1	1.14×10^6	1.12×10^6	1.50×10^6	110×10^6	1.44×10^6
2	1.98×10^6	2.00×10^6	3.65×10^6	1.65×10^6	1.55×10^6
3	3.28×10^6	2.40×10^6	4.77×10^6	1.70×10^6	2.30×10^6
4	2.80×10^6	1.92×10^6	4.82×10^6	1.60×10^6	2.45×10^6

a. Values are mean ± standard deviation.

4. Discussion

4.1. pH Dynamics during Fermentation of Agricultural Residues

The pH profile observed during the fermentation of the different lignocellulosic substrates provides important insights into microbial metabolism, substrate composition, and the overall stability of the fermentation system. Across all substrates, the fermentation environment remained within a slightly alkaline to near-neutral range (pH 6.0 - 9.1), which is atypical for conventional ethanol fermentation systems that often operate under mildly acidic conditions (pH 4.0 - 5.5). This deviation can be attributed to the alkaline pre-treatment applied to the substrates prior to fermentation, which likely resulted in residual buffering capacity despite extensive washing or it could be as a result of factors otherwise unknown to the authors.

Cassava peels exhibited a progressive decrease in pH from 8.0 on day 1 to 6.0 by day 4, indicating increased metabolic activity and organic acid production dur-

ing fermentation. The decline in pH may be associated with the conversion of fermentable sugars into ethanol and secondary metabolites such as organic acids, carbon dioxide, and other by-products of yeast metabolism. Similar reductions in pH during lignocellulosic fermentation have been reported by Mood *et al.* [16], who attributed pH decline to microbial assimilation of sugars and accumulation of acidic intermediates.

In contrast, groundnut shells demonstrated a continuous increase in pH, reaching 9.1 by day 4. This alkaline shift may reflect the lower availability of fermentable carbohydrates and higher lignin content of groundnut shells, which can limit yeast metabolic activity and reduce acid production. Additionally, protein degradation and ammonia release from nitrogenous compounds present in groundnut shells may have contributed to the observed pH increase. Similar alkaline trends during fermentation of nitrogen-rich agricultural residues have been documented by Saini *et al.* [17].

Corn cob, rice straw, and sawdust showed relatively stable pH values with only minor fluctuations, suggesting a balance between acid production and buffering effects of residual alkali. Stable pH conditions are advantageous for yeast survival and metabolic consistency, particularly in non-optimized fermentation systems. According to Zabed *et al.* [18], pH stability during fermentation enhances yeast viability and prevents premature inhibition of enzymatic pathways involved in ethanol biosynthesis.

Overall, the pH dynamics observed in this study underscore the strong influence of substrate composition and pre-treatment chemistry on fermentation behavior. While the alkaline pH range did not completely inhibit *Saccharomyces* growth, it likely contributed to reduced ethanol yields compared to conventional acidic fermentation systems.

4.2. Trends in Reducing Sugar Utilization during Fermentation

Reducing sugar concentration is a critical indicator of substrate hydrolysis efficiency and microbial sugar uptake during fermentation. The results showed considerable variation in initial reducing sugar concentrations among substrates, reflecting differences in carbohydrate composition, lignocellulosic complexity, and pre-treatment effectiveness.

Rice straw recorded the highest initial reducing sugar concentration on day 1 (0.076 g/g), indicating effective alkaline pre-treatment and partial hydrolysis of hemicellulose and cellulose fractions. Rice straw is known to contain a relatively high proportion of hemicellulose, which is more readily solubilized under alkaline conditions, leading to increased release of fermentable [19]. However, a sharp decline in reducing sugar concentration over subsequent days suggests rapid microbial consumption and possibly limited sustained hydrolysis during fermentation.

Corn cob exhibited the lowest reducing sugar concentrations throughout the fermentation period, with values declining to near depletion by day 4. This trend suggests that although corn cob is a cellulose-rich substrate, the degree of hydrol-

ysis achieved may have been insufficient to release substantial quantities of fermentable sugars. The crystalline structure of cellulose and residual lignin may have limited enzyme accessibility, even after alkaline pre-treatment. Similar findings were reported by Chandel *et al.* [20], who observed low sugar yields from inadequately hydrolyzed corn cob substrates.

Cassava peels maintained relatively stable reducing sugar concentrations, with only moderate declines during fermentation. This behavior reflects the inherently high starch content of cassava residues, which are more readily converted into fermentable sugars compared to lignocellulosic biomass. The sustained availability of reducing sugars likely contributed to enhanced yeast growth and ethanol production observed in cassava peel fermentations. Studies by Ajao *et al.* [21] have demonstrated that cassava-based substrates consistently outperform many agricultural residues in terms of sugar availability and fermentation efficiency.

Sawdust and groundnut shells showed low to moderate reducing sugar concentrations with minor fluctuations, indicating limited sugar release and slower microbial uptake. The high lignin content of these substrates restricts enzymatic hydrolysis and sugar liberation, thereby reducing fermentability. According to Kumar *et al.* [22], substrates with lignin content exceeding 25% often require more aggressive pre-treatment strategies to achieve economically viable sugar yields.

The overall decline in reducing sugar concentrations across all substrates confirms active microbial utilization during fermentation. However, the relatively low absolute sugar values highlight the need for improved hydrolysis strategies, such as enzymatic saccharification or combined pre-treatment methods, to enhance fermentable sugar availability.

4.3. Ethanol Production Patterns during Fermentation

Ethanol production trends closely mirrored the availability and utilization of reducing sugars across the different substrates. Cassava peels consistently produced the highest ethanol concentrations, achieving a peak value of 0.045 g/g on day 1, followed by a gradual decline. This early peak suggests rapid fermentation of readily available sugars, characteristic of starch-rich substrates. Similar rapid ethanol production from cassava residues has been widely reported in the literature [23].

Groundnut shells and sawdust exhibited delayed peak ethanol production, reaching maximum values on day 2. This pattern suggests slower sugar release and gradual microbial adaptation to the substrates. The delayed fermentation kinetics may reflect the time required for yeast cells to access and metabolize limited fermentable sugars derived from complex lignocellulosic structures. Zabed *et al.* [18] noted that substrates with high lignin content often exhibit prolonged lag phases and delayed ethanol peaks.

Corn cob and rice straw showed comparatively lower ethanol yields, with concentrations declining steadily after day 1. This trend may be attributed to limited sugar availability, suboptimal pH conditions, and possible accumulation of inhibitory compounds such as phenolics generated during alkaline pre-treatment. Phe-

nolic inhibitors are known to impair yeast metabolism by disrupting cell membranes and enzyme activity [24].

The decline in ethanol concentration observed by day 4 across all substrates may also indicate ethanol volatilization, microbial re-assimilation, or metabolic shifts toward maintenance energy rather than growth-associated ethanol production. Additionally, prolonged exposure to alkaline conditions may reduce yeast fermentation efficiency over time. According to Walker and Walker [25], *Saccharomyces* species exhibit optimal ethanol productivity under mildly acidic conditions, and deviations can result in reduced ethanol accumulation.

Despite these limitations, the ethanol concentrations achieved in this study demonstrate the feasibility of converting diverse agricultural residues into bioethanol using simple pre-treatment and fermentation techniques. However, optimization of pH control, hydrolysis efficiency, and fermentation duration is necessary to improve overall yields.

4.4. Growth Dynamics of *Saccharomyces* Species during Fermentation

The growth profile of *Saccharomyces* species (PWA) provides valuable insights into microbial adaptation, substrate suitability, and fermentation performance. Across all substrates, yeast populations increased from day 1 to day 3, indicating active cell division and metabolic engagement with the substrates.

Cassava peels consistently supported the highest yeast cell densities, reaching a maximum of 4.82×10^6 CFU/mL by day 4. This superior growth performance can be attributed to the high availability of fermentable carbohydrates, favorable nutrient composition, and reduced structural complexity of cassava peels. Enhanced yeast growth on cassava-based substrates has been reported by Adewumi *et al.* [26], who highlighted the role of starch accessibility in promoting microbial proliferation.

Corn cob and rice straw supported moderate yeast growth, with peak populations observed on day 3 followed by a slight decline. The reduction in cell density may reflect nutrient depletion, accumulation of metabolic by-products, or suboptimal pH conditions. The stabilization or decline of yeast populations during late fermentation stages is a common phenomenon and has been linked to ethanol toxicity and nutrient limitation [27].

Sawdust exhibited the lowest yeast counts throughout the fermentation period, underscoring its limited fermentability. The dense lignin matrix and low carbohydrate content likely restricted microbial access to fermentable substrates, resulting in poor growth performance. Groundnut shells showed intermediate growth patterns, suggesting partial suitability as a fermentation substrate when compared to sawdust.

The correlation between yeast growth, reducing sugar consumption, and ethanol production observed in this study reinforces the central role of substrate composition in determining fermentation efficiency. Substrates that supported higher

yeast densities also produced higher ethanol concentrations, highlighting the importance of selecting carbohydrate-rich and structurally accessible feedstocks for bioethanol production.

4.5. Implications for Sustainable Bioethanol Production

The findings of this study demonstrate that agricultural residues differ significantly in their suitability for bioethanol production, with cassava peels emerging as the most promising substrate among those evaluated. The ability to convert locally available agro-wastes into bioethanol has important implications for waste management, renewable energy generation, and rural economic development, particularly in agrarian regions such as Benue State, Nigeria.

However, the relatively low ethanol yields and sugar concentrations observed indicate the need for further process optimization. Incorporation of enzymatic saccharification, improved pre-treatment protocols, pH control, and strain improvement could substantially enhance fermentation performance. According to Kumar *et al.* [28], integrating biological and physicochemical pre-treatment strategies is critical for achieving commercially viable bioethanol yields from lignocellulosic biomass.

From a policy and development perspective, the valorization of agricultural residues for bioethanol production can support multiple objectives: rural job creation, waste management, reduction of open burning or uncontrolled disposal, and partial substitution of imported liquid fuels. Nigeria currently operates below its potential for bioethanol production; historic estimates suggest an installed capacity far below national demand, underscoring the opportunity for expanded domestic production if technical bottlenecks are resolved. Advancing second-generation bioethanol projects will require coordinated investments in feedstock collection logistics, pre-treatment and hydrolysis infrastructure, and supportive policies to encourage private sector and community-scale initiatives [4].

5. Conclusion

In summary, the fermentation study demonstrated that NaOH-pre-treated agricultural residues can support yeast growth and produce measurable ethanol, but yields were constrained by limited saccharification, alkaline fermentation pH and likely the presence of inhibitory compounds. Rice straw and cassava peels showed particular promise in terms of early sugar release and ethanol formation, respectively, but all substrates would benefit from integrated pre-treatment-hydrolysis-detoxification optimization. Implementing these recommended processes and conducting techno-economic assessments will help determine the feasibility of scaling up bioethanol production in Benue State and Nigeria at-large.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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