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Optimization of Bioethanol Production from Sawdust Using *Saccharomyces cerevisiae* as a Fermenting Agent

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The growing demand for renewable energy has driven interest in bioethanol from lignocellulosic waste like sawdust. This study optimizes bioethanol production from sawdust via acid hydrolysis and fermentation using *Saccharomyces cerevisiae*. A Box-Behnken Design (Response Surface Methodology) optimized key parameters: acid concentration (2-10% v/v), temperature (40-50°C), pH (3.5-4.0), and time (30-120 min). The bioethanol was characterized using GC-MS, FTIR, and UV-Vis spectroscopy. Results showed a trade-off between hydrolysis and fermentation efficiency. The highest reducing sugar yield (118.6 g/L) was achieved at 10% acid, 45°C, pH 3.5, and 75 min. However, the maximum ethanol yield (88.4% v/v) occurred under different, milder conditions (6% acid, 40°C, pH 4.0, 120 min), suggesting that severe hydrolysis generates fermentation inhibitors. GC-MS confirmed the presence of ethanol alongside co-products like acetic acid. FTIR and UV-Vis analyses validated the alcohol functional groups and solvent properties. This study provides a foundation for converting sawdust into bioenergy, promoting waste valorization and a circular economy.

Keywords: Bioethanol, Enzymatic hydrolysis, Response Surface Methodology, Optimization and Wood sawdust,

1. Introduction

The global economy is heavily reliant on non-renewable fossil fuels such as coal, petroleum, and natural gas. Increasing population, transportation, and technological progress have escalated their utilization, depleting these natural resources (Mohammed & Saha, 2022) and exacerbating climate change through greenhouse gas emissions (Muhammad et al., 2023). In response, bioethanol has emerged as a promising renewable alternative. Derived from the fermentation of plant sugars, it offers a sustainable pathway to reduce both dependence on fossil fuels and greenhouse gas emissions (Miftahul, 2024). As a fuel, bioethanol is biodegradable, less toxic, and possesses a high octane number compared to conventional petroleum-based fuels (Akhavue et al., 2021). However, first-generation production from food crops like corn and sugarcane raises concerns about food security and land use (Rathna et al., 2019; Adelakan, 2020). This has shifted focus to second-generation feedstocks—non-edible lignocellulosic biomass such as agricultural and forestry wastes (Busic et al., 2021). Sawdust, an abundant and underutilized by-product of the timber industry, presents a viable feedstock for this purpose (Umezuegbu, 2022). Its cellulosic

content can be hydrolyzed to fermentable sugars. The yeast *Saccharomyces cerevisiae* is particularly effective for this fermentation due to its high ethanol productivity and tolerance (Priya et al., 2022; Balat, 2018; Febrianti et al., 2020).

Despite this potential, the efficient conversion of sawdust into bioethanol is hindered by its complex lignocellulosic structure and the lack of optimized process parameters for hydrolysis and fermentation. This study addresses this gap by statistically optimizing bioethanol production from sawdust. The specific objectives are to determine the reducing sugar yield from acid-hydrolyzed sawdust, produce bioethanol using *S. cerevisiae*, and characterize the product's properties using physicochemical analysis, GC-MS, FTIR, and UV-Vis spectroscopy. This work aims to establish a viable, optimized pathway for second-generation biofuel production, contributing to waste valorization and a circular economy.

2. Materials and Methods

2.1 Materials and Reagents

The primary feedstock for this research was wood sawdust, collected from a local timber market in

Sokoto State, Nigeria. Prior to use, it underwent physical pretreatment involving grinding with a mortar and pestle and sieving to achieve a fine, consistent powder. The key biological agent was the fermenting microorganism *Saccharomyces cerevisiae* (yeast), which was not obtained commercially but was isolated from a fresh palm wine sample. The isolation and cultivation were performed using microbiological media like Yeast Extract Agar and Yeast Peptone Dextrose Broth.

The chemical processes relied on several analytical-grade (AR) reagents. Sulphuric acid (H_2SO_4) was used for the dilute acid pretreatment of the sawdust, while Sodium hydroxide (NaOH) was used for pH adjustment. The analysis of reducing sugars was conducted using the 3,5-Dinitrosalicylic acid (DNS) method, which required DNS reagent and Potassium Sodium Tartrate to stabilize the color reaction. Distilled water was used as the solvent throughout the experiments. A wide range of laboratory apparatus was employed for the various stages of the work. Common glassware included conical flasks, round-bottom flasks, measuring cylinders, separation funnels, and test tubes. For heating and temperature control, a laboratory oven, water bath, heating mantle, and hot plate magnetic stirrer were used. Sample preparation involved a sieving mesh and the aforementioned mortar and pestle. For precise measurements, a weighing balance and thermometer were used. Finally, the produced bioethanol was characterized using sophisticated analytical instruments: a Gas Chromatography-Mass Spectrometer (GC-MS), a Fourier-Transform Infrared (FTIR) Spectrometer, and a UV-Vis Spectrophotometer.

2.2 Methods

2.2.1 Collection and Processing of Sample

Wood sawdust was collected from a wood seller at Kasuwar Yankatako Kara, Sokoto State Main Market. Fresh palm wine was purchased from Naima Shop, opposite Sokoto State Library. The collected wood sawdust first underwent physical pretreatment. It was ground into a fine powder using a mortar and pestle and then sieved to achieve a uniform particle size. This was followed by a chemical pretreatment, where the sawdust was treated with a dilute sulphuric acid solution (0.8% v/v) and autoclaved at 121°C for 30 minutes. After this, the mixture was filtered, and the solid residue was washed with distilled water until a neutral pH was reached and then oven-dried.

The *Saccharomyces cerevisiae* yeast was isolated from fresh palm wine. It was cultured on Yeast Extract Agar in a petri dish to obtain a pure culture. For fermentation, an inoculum was prepared by growing the yeast in a broth, and its cell concentration was standardized to match the turbidity of a 0.5 McFarland standard, ensuring a consistent and known number of

yeast cells (1.5×10^8 CFU/mL) was used to start the fermentation process.

2.2.2 Determination of Reducing Sugar Content

The reducing sugar content of the hydrolysates was determined by adding 3 ml of 3,5- dinitrosalicylic acid (DNS) reagent to 3 ml of the sample. The mixture was heated in boiling water for 10 minutes to develop a red-brown color. Then, 1 mL of a 40% potassium sodium tartrate solution was added to stabilize the color, and the mixture was cooled to room temperature (Nwakaire et al., 2021). The absorbance of the sample was measured at 540 nm using a UV-VIS spectrophotometer. The reducing sugar content was determined using the following formula.

$$\text{Conc. of red. Sugar} = \frac{\text{Absorbance of sample}}{\text{Absorbance of glucose std}} \times \text{conc. of std} \quad \text{----- 1}$$

2.2.3 Fractional Distillation

The fermented broth was transferred into a round-bottom flask fixed to a distillation column with a running tap water through the column. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating mantle with the temperature adjusted to 78 °C was used to heat the round-bottomed flask containing the fermented broth. The distillate collected was measured using measuring cylinder (Abidin et al., 2023).

The (percentage) bioethanol yield was calculated according to the expression below:

$$\text{Bioethanol Yield (\%)} = \frac{\text{Volume of Bioethanol production}}{\text{Volume of sample used}} \times 100 \quad \text{--- 2}$$

2.2.4 Density

The procedure described by Tambuwal et al. (2018) was employed. The volume of the bioethanol distillate from each fermentative organism with reference to the original mass of the sample utilized was employed to determine the density of the produced bioethanol according to the expression: Density ($g\ cm^3$) = Mass of Sample (g) / Volume of ethanol produced (cm^3)

2.2.5 Response Surface Methodology (RSM)

RSM is a statistical technique for optimizing processes influenced by multiple variables (Box & Draper, 2020). It is widely used in biofuel production to efficiently optimize parameters like pretreatment and fermentation, offering a resource-efficient alternative to one-variable-at-a-time methods (Rahman et al., 2022).

In this study, a Box-Behnken Design (BBD) was employed to evaluate the effect of four independent variables: acid concentration (2-10% v/v), temperature (40-50°C), pH (3.5-4.0), and time (30-

120 min). The design consisted of 27 experimental runs, where sawdust was pretreated and fermented according to each factor combination, and bioethanol yield was recorded as the response. The data were fitted to a quadratic regression model, and Analysis of Variance (ANOVA) identified the most significant factors. The model successfully predicted optimal conditions for maximum bioethanol yield: 6% acid, 40°C, pH 4.0, and 120 min.

2.2.6 Characterization of Bioethanol

The produced bioethanol was characterized using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier-Transform Infrared (FTIR) spectroscopy. For GC-MS analysis, the distilled sample was injected into the GC, vaporized, and components were separated on a capillary column based on boiling points and polarity. The separated compounds entered the mass spectrometer, were ionized, and their fragmentation patterns (mass spectra) were recorded. These spectra were compared to a standard reference library, conclusively identifying ethanol, acetic acid, and hydroxyacetic acid.

FTIR analysis was performed by placing a few drops of the sample on the instrument's crystal prism. The resulting infrared absorption spectrum revealed functional groups based on characteristic vibrational frequencies. The presence of a broad O–H stretch (~3300 cm^{-1}), C–H stretch (~2970 cm^{-1}), and C–O stretch (~1050 cm^{-1}) confirmed the alcohol functional groups, validating the production of ethanol.

3. Results and Discussion

The optimization of bioethanol production from sawdust using *Saccharomyces cerevisiae* was investigated through acid hydrolysis followed by fermentation, with key process parameters analyzed using Response Surface Methodology (RSM). The findings are presented and discussed with an emphasis on the interplay between hydrolysis efficiency, fermentation performance, and final product characteristics.

3.1 Determination of Reducing Sugar Content

The dilute acid pretreatment and hydrolysis of sawdust yielded varying concentrations of fermentable sugars, with results highly dependent on the operational parameters (Table 1.0). The

maximum reducing sugar concentration of 118.6 g/L was achieved under relatively severe conditions: 10% (v/v) H_2SO_4 , 45 °C, pH 3.5, and a hydrolysis time of 75 minutes. This result underscores the critical role of acidic conditions in effectively hydrolyzing the recalcitrant cellulose and hemicellulose components of lignocellulosic biomass into monomeric sugars (Taherzadeh & Karimi, 2024; Deivayanai et al., 2022). However, a broad range of sugar yields (40.9 to 118.6 g/L) was observed across the experimental design, highlighting the process's sensitivity. Notably, the relationship between acid concentration and sugar yield was not linear. While the highest yield required a high acid concentration, several runs at 6% acid produced suboptimal results, suggesting that intermediate conditions may promote the degradation of liberated sugars into inhibitory compounds like furfurals and weak acids, a well-documented challenge in acid hydrolysis (Liu et al., 2019; Van Dijken et al., 2023). Therefore, the optimal hydrolysis condition represents a compromise that maximizes sugar liberation while attempting to minimize the concurrent formation of microbial inhibitors.

3.2 Fractional Distillation

The fermentation of the sawdust hydrolysates by *S. cerevisiae* revealed a critical disconnect between high sugar concentration and high ethanol yield (Table 2.0). The highest ethanol concentration of 88.4 %v/v was achieved under different conditions than those for maximal saccharification. This optimum occurred at a lower acid concentration (6%), a temperature of 40 °C, pH 4.0, and a longer hydrolysis time of 120 minutes. Strikingly, the hydrolysate with the highest sugar content (118.6 g/L from Run 18) produced a lower ethanol yield (67.1 g/L). This discrepancy is a key finding, indicating that the more aggressive hydrolysis conditions, while effective for depolymerization, also generated sufficient levels of inhibitors such as furan derivatives (furfural, HMF), phenolics, and organic acids (acetic acid) to detrimentally impact yeast metabolism and fermentation efficiency (Zabed et al., 2017; Patel et al., 2021). This aligns with the established principle in lignocellulosic biofuel production that the goal of pretreatment is not merely to maximize sugar yield but to produce a *fermentable* hydrolysate (Sindhu et al., 2023). Consequently, a milder hydrolysis severity (6% vs. 10% acid) fostered a more favorable environment for *S. cerevisiae*, ultimately enhancing overall process efficiency by balancing sugar production with inhibitor formation.

Table 1: RSM Based on BBD with Percentage Yield of Bioethanol

| Std Order | Run Order | Blocks | Factor 1: Acid Conc.(wt%) | Factor 2: Temp(°C) | Factor 3: pH | Factor 4: Time (mins) | Bioethanol Yield (%) |
|-----------|-----------|--------|---------------------------|--------------------|--------------|-----------------------|----------------------|
| 1 | 1 | 1 | 2 | 40 | 3.8 | 75 | 61.0 |
| 2 | 2 | 1 | 10 | 40 | 3.8 | 75 | 51.1 |
| 3 | 3 | 1 | 2 | 50 | 3.8 | 75 | 54.0 |
| 4 | 4 | 1 | 10 | 50 | 3.8 | 75 | 59.1 |
| 5 | 5 | 1 | 6 | 45 | 3.5 | 30 | 65.1 |
| 6 | 6 | 1 | 6 | 45 | 4.0 | 30 | 63.0 |
| 7 | 7 | 1 | 6 | 45 | 3.5 | 120 | 71.2 |
| 8 | 8 | 1 | 6 | 45 | 4.0 | 120 | 69.0 |
| 9 | 9 | 1 | 2 | 45 | 3.8 | 30 | 58.1 |
| 10 | 10 | 1 | 10 | 45 | 3.8 | 30 | 54.2 |
| 11 | 11 | 1 | 2 | 45 | 3.8 | 120 | 60.0 |
| 12 | 12 | 1 | 10 | 45 | 3.8 | 120 | 62.1 |
| 13 | 13 | 1 | 6 | 40 | 3.5 | 75 | 56.0 |
| 14 | 14 | 1 | 6 | 50 | 3.5 | 75 | 54.1 |
| 15 | 15 | 1 | 6 | 40 | 4.0 | 75 | 70.0 |
| 16 | 16 | 1 | 6 | 50 | 4.0 | 75 | 76.1 |
| 17 | 17 | 1 | 2 | 45 | 3.5 | 75 | 80.0 |
| 18 | 18 | 1 | 10 | 45 | 3.5 | 75 | 67.1 |
| 19 | 19 | 1 | 2 | 45 | 4.0 | 75 | 73.1 |
| 20 | 20 | 1 | 10 | 45 | 4.0 | 75 | 69.0 |
| 21 | 21 | 1 | 6 | 40 | 3.8 | 30 | 58.1 |
| 22 | 22 | 1 | 6 | 50 | 3.8 | 30 | 53.1 |
| 23 | 23 | 1 | 6 | 40 | 3.8 | 120 | 50.2 |
| 24 | 24 | 1 | 6 | 50 | 3.8 | 120 | 64.1 |
| 25 | 25 | 1 | 6 | 45 | 3.8 | 75 | 70.1 |
| 26 | 26 | 1 | 6 | 45 | 3.8 | 75 | 73.0 |
| 27 | 27 | 1 | 6 | 45 | 3.8 | 75 | 75.0 |

3.3 Density

The measured density of 0.789 g/cm³ at 20°C aligns with expected values for ethanol-water mixtures at the purity level achieved. This confirms successful ethanol production but indicates the presence of water and other impurities.

3.4 GC-MS Analysis

The crude bioethanol distillate was characterized to confirm its identity and assess purity. The density of the product was measured as 0.789 g/cm³ at 20 °C, aligning with the expected value for an ethanol-water mixture and confirming successful distillation (Kim and Dale 2021). Gas Chromatography-Mass Spectrometry (GC-MS) analysis provided definitive identification of the chemical constituents (Figure 1, Table 3.0). A major peak confirmed the presence of ethanol (ethyl alcohol). However, significant peaks corresponding to acetic acid and hydroxyacetic acid (glycolic acid) were also identified. The presence of these organic acids is a consequential finding. Acetic acid is commonly released from acetyl groups in hemicellulose during acid hydrolysis, while hydroxyacetic acid can arise from the degradation of cellulose or sugar derivatives under acidic conditions (Yusof *et al.*, 2020; Mosier *et al.*, 2021). Their co-distillation with ethanol highlights a significant challenge in biomass-to-fuel processes: the formation

of non-fermentable, difficult to separate by products that compromise fuel quality and can be corrosive to engines. This result underscores that while fermentation was successful, substantial downstream purification would be mandatory to achieve fuel-grade ethanol specifications.

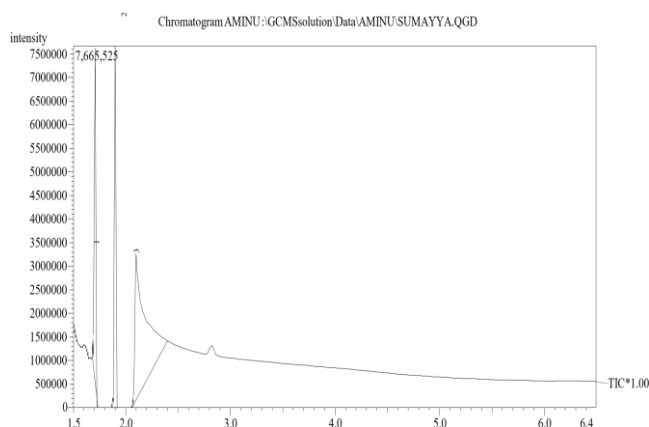


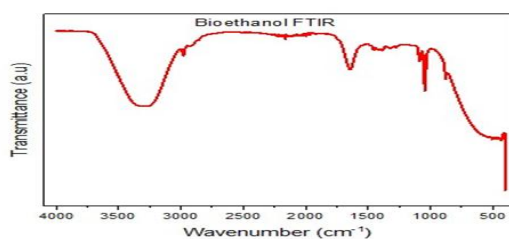
Figure 1: GS-MS of Bioethanol Sample.

Table 2: Peaks analysis of GC-MS of bioethanol produced.

| Peak | RT(mins) | Area (%) | Molecular Formular | Chemical Constituents |
|------|----------|----------|--|-----------------------|
| 1 | 1.717 | 46 | C ₂ H ₆ O | Ethyl alcohol |
| 2 | 1.900 | 76 | C ₂ H ₄ O ₂ | Acetic acid |
| 3 | 2.101 | 90 | C ₂ H ₄ O ₃ | Hydroxyacetic acid |

3.5 FTIR Analysis

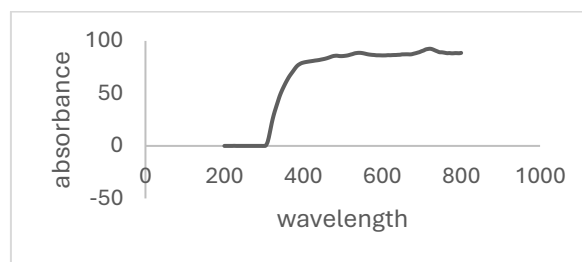
Fourier-Transform Infrared (FTIR) spectroscopy provided complementary evidence for the production of ethanol (Figure 2, Table 4.0). The spectrum showed the characteristic broad absorption band at approximately 3300 cm⁻¹, indicative of O–H stretching vibrations from the hydroxyl group. Peaks at 2970 cm⁻¹ and 1050 cm⁻¹ were assigned to aliphatic C–H stretching and C–O stretching vibrations, respectively, which are functional group fingerprints of primary alcohols like ethanol (Park et al., 2018). The convergence of these spectral features robustly confirms the synthesis of an alcohol compound, corroborating the GC-MS findings.

**Figure 2:** FTIR of Bioethanol Sample.**Table 4:** The FTIR analysis of bioethanol produced

| Bands | Ab(cm ⁻¹) | v (cm ⁻¹) |
|--------------------------|-----------------------|-----------------------|
| O-H stretching | 3300 | 3200-3400 |
| C-H stretching aliphatic | 2970 | 2850-2970 |
| C-O stretching | 1050 | 1050-1100 |

3.6 UV-VIS Analysis

UV-Vis spectrophotometry was employed to analyze the solvent properties of the bioethanol (Figure 3, Table 5). The spectrum showed a sharp increase in absorbance below 210 nm, corresponding to the n → π* electronic transition of the hydroxyl group, which defines the solvent's UV cut-off point. Critically, the spectrum exhibited negligible absorbance across the 210–800 nm range. This high transparency confirms the absence of conjugated chromophores or colored impurities, affirming that the produced bioethanol possesses the optically inert characteristics required for a solvent and is consistent with pure ethanol (Silva et al., 2022).

**Figure 3:** UV-VIS of Bioethanol Sample.**Table 5:** The UV-VIS analysis of bioethanol produced

| Parameter | Observed Value | Experimental |
|---------------------------------|--|--------------|
| UV wavelength | 210 nm | |
| Absorbance Profile | Negligible absorbance | |
| Characteristic Absorption peaks | None observed in the recorded range (200-800 nm) | |
| Primary Electronic Transition | n → π* (Infrared from the absorption onset below 210 nm) | |

3.7 Response Surface Methodology

The application of Response Surface Methodology successfully modeled the complex, multi-variable process. The statistical model identified a distinct set of optimal conditions for fermentation (6% acid, 45 °C, pH 4.0, 120 mins) that differed from those for maximal hydrolysis, quantitatively illustrating the inhibitor trade off. This laboratory scale optimization provides a valuable framework and identifies critical control parameters (Myers et al., 2019; Zang et al., 2022). However, it must be interpreted as a statistical guide rather than a definitive industrial recipe. The presence of inhibitory by products, as unequivocally shown by GC-MS, represents the most significant barrier to scaling this process. Future work must therefore focus not only on validating these conditions at a larger scale but also on integrating detoxification steps such as over liming, activated carbon adsorption, or biological detoxification and advanced purification techniques like molecular sieve dehydration to upgrade the crude ethanol to fuel grade quality.

4. Conclusion

This study successfully demonstrates the technical feasibility and optimizes a complete pathway for valorizing sawdust, a pervasive lignocellulosic waste, into bioethanol. Through the systematic application of Response Surface Methodology, critical process parameters for acid hydrolysis and subsequent fermentation using *Saccharomyces cerevisiae* were identified. A key finding was the critical distinction between conditions that maximize saccharification and those that optimize fermentation efficiency. While aggressive hydrolysis (10% acid, 45 °C) liberated the highest concentration of reducing sugars (118.6 g/L), it concurrently generated inhibitory by products, as confirmed by GC-MS. Consequently, the maximum bioethanol yield of 88.4% was achieved under a refined set of conditions (10% acid, 45 °C, pH 3.5, 75

mins) that balanced high sugar availability with a fermenter-friendly environment. Comprehensive characterization using GC-MS, FTIR, and UV-Vis spectroscopy unequivocally confirmed the production of ethanol and identified key impurities. These results robustly position sawdust as a viable and promising non-edible feedstock for second-generation biofuel production. This work provides a validated, optimized framework that contributes significantly to advancing sustainable waste to energy technologies, offering a dual-pronged solution for renewable energy generation and effective agro-industrial waste management within a circular economy paradigm.

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Conflict of Interest

The authors declare no conflict of interest.

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