

Alkaline Pre-treatment of *Gmelina arborea* Leaves and Bark for Enhanced Bioethanol Production

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ABSTRACT

Lignocellulosic biomass from *Gmelina arborea* leaves and bark represents a promising non-edible feedstock for second-generation bioethanol, addressing food-fuel conflicts and environmental concerns associated with fossil fuels. The structural complexity of lignocellulose limits the accessibility of fermentable sugars during hydrolysis. Pre-treatment methods such as alkaline treatment are widely employed to disrupt lignin structures and enhance cellulose availability. This study investigated the effectiveness of alkaline pre-treatment using sodium hydroxide (NaOH) on the leaves and bark of *Gmelina arborea* for bioethanol production. Biomass samples were treated with 2.0 M, 2.5 M, and 3.0 M NaOH solutions for 24 hours at room temperature, washed, neutralized to pH 4–6 with 1 M H₂SO₄, and fermented with *Saccharomyces cerevisiae* for 5 days under anaerobic conditions. Ethanol was recovered by distillation (78 °C) and purified by rotary evaporation. The highest ethanol yield (25 ml) was obtained from leaf biomass pre-treated with 2.5 M NaOH, while bark biomass yielded the highest ethanol volume (18 ml) at 3.0 M NaOH. Lower concentrations (2.0 M) produced lower yields but higher purity (densities as low as 0.724 ± 0.013 g/cm³ for bark). Ethanol density ranged from 0.724 ± 0.013 to 0.952 ± 0.01 g/cm³. Residue fermentation yielded no ethanol, confirming the necessity of hydrolysate processing. Alkaline pre-treatment improved ethanol recovery from both tissues, with biomass-specific optima (2.5 M for leaves; 3.0 M for bark). *Gmelina arborea* residues therefore warrant further investigation as a second-generation bioethanol feedstock, provided yields are confirmed by sugar quantification and mass-balance calculations in future work.

Keywords: *Gmelina arborea*, alkaline pre-treatment, lignocellulosic biomass, bioethanol yield, ethanol density

1.0 INTRODUCTION

Globally, the demand for renewable energy sources has intensified due to the depletion of fossil fuels and increasing environmental concerns (Holechek *et al.*, 2022). Bioethanol that is derived from lignocellulosic biomass has become a promising alternative fuel because it is renewable, sustainable, and capable of reducing greenhouse gas emissions (Igwebuike *et al.*, 2024). Primarily, lignocellulosic biomass consists of cellulose, hemicellulose, and lignin, which form a complex structure that resists enzymatic degradation. This structural recalcitrance limits the release of sugars that is fermentable and required for ethanol production. Consequently, pre-treatment processes are necessary to break down lignin structures and increase cellulose accessibility (Ale *et al.*, 2026). *Gmelina arborea*, a fast-growing deciduous tree widely planted in Nigeria (over 60% of planted forests), sheds leaves during the dry season and generates bark waste during harvesting, making both parts ideal non-edible feedstocks (Umar *et al.*, 2024). Conventional fossil fuels contribute significantly to greenhouse gas emissions and energy insecurity; bioethanol from lignocellulose can mitigate these issues by reducing CO₂ emissions and enhancing energy security (El-Araby, 2024).

However, the complex structure of lignocellulose comprising cellulose (40–50%), hemicellulose (20–30%), and lignin (15–25%) requires effective pre-

treatment to disrupt recalcitrant bonds and expose cellulose for enzymatic or microbial hydrolysis (Prasad *et al.*, 2023). Among the various pre-treatment techniques, alkaline pre-treatment has proven effective in solubilizing lignin and disrupting lignocellulosic matrices. Alkaline agents such as sodium hydroxide break ester linkages in lignin and hemicellulose, thereby enhancing enzymatic hydrolysis and sugar release (Baksi *et al.*, 2023). This study evaluates the effectiveness of sodium hydroxide pre-treatment on *Gmelina arborea* leaves and bark for bioethanol production, focusing on the influence of NaOH concentration on ethanol yield and purity.

2.0 MATERIALS AND METHODS

2.1 Study Area

The samples were collected from the Nigerian Defence Academy (NDA), Kaduna, Nigeria, located at approximately 10.5291°N latitude and 7.4383°E longitude. The area lies within the Guinea Savannah ecological zone, characterized by moderate temperatures and seasonal rainfall (Adeaga *et al.*, 2022).

2.2 Sample Collection and Proximate Composition

Mature leaves and bark of *Gmelina arborea* were collected from trees on the Nigerian Defence Academy campus, Kaduna. Samples were air-dried for two weeks at ambient temperature, ground to a fine particle

size, and stored in sterile containers. Proximate analysis was performed according to standard AOAC (2007) methods.

2.3 Alkaline Pre-Treatment

Alkaline pre-treatment was carried out using sodium hydroxide (NaOH) solutions at concentrations of 2.0 M, 2.5 M, and 3.0 M. Ten grams (10g) of dried biomass (leaf and bark) were placed in conical flasks containing 100 ml of NaOH solution. The mixtures were allowed to soak for 24 hours at room temperature. After pre-treatment, the mixture was filtered using muslin cloth, and the residues were washed three times with distilled water to remove residual chemicals (Ojo & City., 2023).

2.4 Neutralization

The hydrolysate was adjusted to pH 4–6 with 1 M H₂SO₄, a range suitable for *S. cerevisiae* (Shirvanyan *et al.*, 2025).

2.5 Preparation of Inoculums for Fermentation

Commercial baker's yeast (*Saccharomyces cerevisiae*) was activated by suspending 5g of dried yeast in 30 ml of sterile distilled water that had been boiled for 20 minutes and cooled to 45–50°C. The suspension was mixed thoroughly and held for 10 minutes. Fresh inoculum was prepared immediately for fermentation (Fang *et al.*, 2023).

2.6 Hydrolysate Fermentation

Fermentation was conducted using *Saccharomyces cerevisiae*. The activated baker's yeast inoculum (10 mL) was added to 100 mL hydrolysate in anaerobic flasks. Fermentation proceeded for 5 days at room temperature under anaerobic conditions to facilitate the conversion of fermentable sugars into ethanol (Dawson & Boopathy, 2007).

2.7 Residue Fermentation

The pre-treated samples were used in the fermentation experiment. Exactly 5g of the pre-treated biomass was weighed and added into anaerobic flasks containing 100mL of distilled water and 10 ml of the inoculum (Dawson and Boopathy, 2007). The flasks were shaken vigorously and sealed with aluminium foil and fermentation was allowed to proceed for 5 days at room temperature.

2.8 Ethanol Recovery

No ethanol was recovered for the residue fermentation. For the hydrolysate fermentation, ethanol was recovered by distillation at 78 °C and further concentrated by rotary evaporation (78 °C, 110 rpm). Volume was measured directly. Density was calculated as mass/volume (g/cm³). Residue remaining after filtration of the pre-treated slurry was also fermented under identical conditions as a negative control. The volume and density of the produced ethanol were measured to evaluate yield and purity (Tenkolu *et al.*, 2024).

Volume was measured using a measuring cylinder, and density calculated as:

Density (g/cm³) = mass (g) / volume (cm³) (Anyahoha, 2010).

Yield (g/L) was determined by multiplying volume by ethanol density (0.789 g/cm³) and expressed per 100 g dry substrate (Theodore, 2022).

3.0 RESULTS

3.1 Proximate Composition

The proximate composition of *Gmelina arborea* leaves and bark is presented in Table 1. Leaves contained higher moisture (6.50%), lipid (3.90%), crude fibre (9.10%) and protein (2.40%) than bark. Ash contents were similar (7.50% in leaves; 7.0% in bark). Bark had a slightly higher carbohydrate content (75.40%) than leaves (71.50%).

Table 1: Proximate Composition of Leaf and Bark of *Gmelina Arborea*

Parameter (%)	Leaf	Bark
Moisture	6.50	5.30
Ash content	7.50	7.00
Lipid	2.90	1.90
Crude fibre	9.10	8.40
Protein	2.40	2.00
Carbohydrate	71.50	75.40

3.2 Ethanol Yield from Alkaline Pre-Treated Hydrolysate

The ethanol yield obtained from alkaline pre-treatment varied depending on NaOH concentration and biomass type. The highest ethanol yield (25 ml) was recorded in leaf biomass treated with 2.5 M NaOH, while bark

biomass produced its highest yield (18 ml) at 3.0 M NaOH. The density of ethanol ranged from 0.724 ± 0.013 to 0.952 ± 0.01 g/cm³, suggesting relatively high ethanol purity compared with untreated samples.

Table 2: Volume and Density of Ethanol Produced from Alkaline Pre-Treatment of Leaves and Bark of *G. Arborea*

NaOH Concentration	Biomass	Ethanol Volume (ml)	Density (g/cm ³)
2.0 M	Leaf	7.0	0.784
2.0 M	Bark	6.0	0.724
2.5 M	Leaf	25.0	0.952
2.5 M	Bark	8.0	0.920
3.0 M	Leaf	7.0	0.918
3.0 M	Bark	18.0	0.927

4.0 DISCUSSION

The proximate composition of the leaf and bark of *Gmelina arborea* revealed significant differences and similarities in their nutritional content, which can be instrumental to various applications in food science, pharmacology, and environmental sciences. The carbohydrate content of *Gmelina arborea* bark (75.4%) exceeds that of its leaves (71.5%), indicating both parts are potent energy sources, particularly valuable in regions combating malnutrition with energy-dense foods (Muhammad *et al.*, 2025).

The ethanol yield obtained ranged from 6 to 25 ml. The results has demonstrated that alkaline pre-treatment significantly enhances ethanol production from *Gmelina arborea* biomass. Sodium hydroxide pre-treatment disrupts structures of lignin and increases accessibility to cellulose, allowing more efficient hydrolysis and fermentation (Bensah & Mensah, 2013). The highest yield (25 ml) was obtained from leaf biomass pre-treated with 2.5 M NaOH, whereas the lowest yield (6 ml) was recorded for bark pre-treated with 2.0 M NaOH. In general, increasing NaOH concentration improved ethanol production up to an optimum level, presumably by enhancing the availability of fermentable carbohydrates through the disruption of lignin-carbohydrate associations. Pendse *et al.* (2023) similarly reported that pre-treatment solution concentration significantly influences carbohydrate recovery from plant biomass, which consequently affects ethanol production.

The response to NaOH concentration differed between the two biomass types. For leaf biomass, 2.5 M NaOH produced the highest ethanol yield (25 ml), while increasing the concentration to 3.0 M did not result in further improvement. In contrast, bark biomass responded best to 3.0 M NaOH, producing 18 ml of ethanol. This differential response indicates that optimum alkaline pre-treatment conditions depend on the structural characteristics and chemical composition of the biomass (Alawad & Ibrahim, 2024). Alkaline pre-treatment promotes lignin and hemicellulose solubilization and increases cellulose accessibility for subsequent hydrolysis and fermentation (Baksi *et al.*, 2023). Ale *et al.* (2026) similarly observed that alkaline concentration increment can improve cellulose

accessibility, although excessive treatment may cause structural changes that adversely affect subsequent fermentation. Thus, the lower yield at higher concentration in leaf biomass may indicate that 2.5 M NaOH represented a more suitable pre-treatment condition for leaf material.

The density of the bioethanol samples ranged from 0.724 ± 0.013 to 0.952 ± 0.01 , while the broader dataset was reported to range from 0.724 to 1.068 g/L, with the control samples recording the highest densities. The lowest density (0.724 ± 0.013) was observed in leaf biomass treated with 2.5 M NaOH, corresponding to the treatment that also produced the highest ethanol volume (25 ml). The reported densities were compared with the range of 0.796–0.858 g/L reported by Carreon *et al.* (2023), while Ijagbemi *et al.* (2025) reported densities of 0.843 g/ml and 0.793 g/ml for ethanol produced from banana and yam peels, respectively. The lower density observed in some treated samples may indicate a greater ethanol proportion relative to residual non-ethanol components; however, density alone should be interpreted cautiously as a measure of ethanol purity because other dissolved or volatile components can also influence density (Yerizam *et al.*, 2026).

The results therefore demonstrate that alkaline pre-treatment effectiveness was dependent on both NaOH concentration and biomass type. Leaf biomass performed best at 2.5 M NaOH, yielding 25 ml, whereas bark required 3.0 M NaOH to achieve its highest yield of 18 ml. These findings agree with reports that alkaline pre-treatment conditions must be optimized according to biomass characteristics, since the concentration and duration of treatment influence lignin removal, cellulose accessibility and subsequent fermentability (Olatunji *et al.*, 2021). Overall, the findings suggest that moderate alkaline pre-treatment was sufficient for leaf biomass, while bark required a higher concentration, highlighting the importance of biomass-specific optimization of NaOH pre-treatment for efficient bioethanol production.

5.0 CONCLUSION

This study demonstrated that alkaline pre-treatment using sodium hydroxide significantly enhances



bioethanol production from *Gmelina arborea* leaves and bark. The optimal NaOH concentration differed between biomass types, with 2.5 M being most effective for leaves and 3.0 M for bark. The results confirm that alkaline pre-treatment improves carbohydrate accessibility and supports efficient fermentation by *Saccharomyces cerevisiae*. Therefore, *Gmelina arborea* biomass represents a promising feedstock for second-generation bioethanol production.

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