J.Natn.Sci.Foundation Sri Lanka 2026 54 (1): 101 - 111
DOI: http://dx.doi.org/10.4038/jnsfsr.v54i1.12547

RESEARCH ARTICLE

Environmental Science

Process optimization and yield enhancement of bioethanol production from *Musa paradisiaca* peel waste

S Yogendrarajah* **and K Ranganathan**
Department of Botany,Faculty of Science, University of Jaffna, Thirunelvely, Sri Lanka.

Submitted: 26 January 2025; Revised: 18 February 2026; Accepted: 05 March 2026

Abstract: The growing global demand for energy and the depletion of fossil fuels create serious challenges for energy security and increasing environmental problems such as pollution, global warming, rising sea levels, and CO_2 emissions. Switching to low-carbon fuels is necessary to fight climate change. Bioethanol, a renewable fuel made from plants, offers a sustainable way to meet energy needs while reducing environmental impact. This study investigates the use of *Musa paradisiaca* peels, an underutilized agro-waste, for bioethanol production. Different parts of the plant, including pseudostem, leaves, roots, peels, and bunch stalk, were tested. Among these, peels produced the highest levels of reducing sugars and bio-alcohol, making them the most effective carbon substrate for further optimization. Fermentation conditions were optimized step by step by altering one factor at a time while maintaining all other parameters constant. The optimization process involved determining the ideal fermentation time, hydrolysis agent, sulfuric acid concentration, yeast inoculum size, and substrate amount. Under the optimized conditions, a 40-hour fermentation time, the use of 0.75M sulfuric acid as the hydrolysis agent, 100 g/L yeast inoculum, and 75 g/100 mL substrate concentration resulted in a significant 8.15-fold increase in bio-alcohol yield compared to the non-optimized conditions. Analysis by GC revealed that the amount of ethanol in the crude alcohol sample was 89.6%. This study shows an effective way to convert banana peels into high purity bioethanol, supporting sustainable waste management and circular bioeconomy strategies.

Keywords: Bioethanol production, fermentation, *Musa paradisiaca*, *Saccharomyces cerevisiae.*

INTRODUCTION

With rising energy demand and the depletion of fossil fuel reserves, transitioning to low-carbon energy sources such as bioethanol has become essential to mitigate climate change and reduce CO_2 emissions (Broda et al., 2022). Bioethanol is produced from renewable, plant-based materials and contributes to energy security by utilizing agricultural residues. Lignocellulosic feedstocks such as rice straw and sugarcane bagasse are commonly used for second generation (2G) bioethanol production. Compared to first generation bioethanol, 2G bioethanol offers greater reductions in greenhouse gas emissions (Danmaliki et al., 2016; Sarkar et al., 2012). More advanced bioethanol technologies, including third-generation algae-based systems and fourth-generation processes involving genetically engineered microorganisms, have been developed to further improve production efficiency and sustainability (Edeh et al., 2021).

Bioethanol production involves pretreatment, hydrolysis, and fermentation. Pretreatment is used to disrupt the lignin structure, thereby improving accessibility to cellulose and hemicellulose. This can be achieved through physical, chemical, biological, or physicochemical methods (Meenakshisundaram et al., 2022; Dimos et al., 2019). Hydrolysis converts

* Corresponding author (sangavee23@gmail.com; https://orcid.org/0009-0007-7215-2045)

complex carbohydrates into fermentable sugars using acid, alkaline, or enzymatic methods (Devi et al., 2016; Binod et al., 2011). Fermentation, mainly carried out by *Saccharomyces cerevisiae*, converts fermentable sugars into ethanol (Hackl et al., 2011). Bioethanol has a high-octane rating, making it suitable for blending with gasoline in spark-ignition engines. However, its low vapor pressure presents challenges that can be addressed by blending it with more volatile fuels (Abdulsalam et al., 2021; Mushimiyimana et al., 2016).

In addition to its use as transportation fuel, bioethanol has wide industrial applications. It is commonly employed in chemical synthesis as a solvent, and also serves as a starting material to produce other biofuels (Yaverino et al., 2024). It acts as an important feedstock for producing bio-based chemicals through polymerization and oxidation, resulting in the formation of industrial plastics and other valuable compounds (Assaf et al., 2024). Bioethanol can be used in power generation, contributing to energy security and diversification of energy sources (Yaverino et al., 2024). Bioethanol is widely utilized in household products, including cleaning agents and personal care items due to its effective solvent properties (Mushimiyimana et al., 2016).

Bioethanol is a renewable energy source produced from agro-waste and other non-edible lignocellulosic biomass, offering an effective approach to waste reduction while minimizing environmental pollution (Mushimiyimana et al., 2016; Yaverino et al., 2024). Its high-octane number enhances engine performance and reduces greenhouse gas emissions, as CO_2 released during combustion is reabsorbed through photosynthesis (Gouthami et al., 2024). Because bioethanol can be produced from agricultural residues, forest residues, and solid waste, it supports sustainability and stimulates local economies (Abdulsalam et al., 2021; Yaverino et al., 2024). However, several challenges remain, including competition with food resources, land use concerns, and environmental impacts associated with crop cultivation and processing (Assaf et al., 2024; Wandscher Busanello et al., 2023).

Bioethanol production from lignocellulosic biomass, such as crop residues and wood, involves various technological challenges. Effective pretreatment methods are required for breaking down complex biomass into fermentable sugars. However, these processes can be costly and technically challenging. The existing technologies for producing second-generation (2G) bioethanol face difficulties in the pretreatment and hydrolysis stages, which can restrict their feasibility for large scale industrial implementation (Yaverino et al., 2024).

Banana cultivation, particularly the Kathali variety (*Musa paradisiaca*), is significant in Sri Lanka's Jaffna peninsula, contributing to the economy and food security. Although bananas are produced in large quantities, much of the plant, including peels, pseudostems, leaves, bunch stalks, and roots, are underutilized or discarded as waste. These parts contain sugars such as glucose, sucrose, and fructose, essential for fermenting bioethanol. Utilizing banana waste for biofuel production can improve waste management, reduce environmental pollution, and promote renewable energy, thereby contributing to sustainability and resource efficiency goals (Pazmiño et al., 2019). Current practices often leave banana residues to decompose in fields, which can lead to disease spread and water pollution Converting this biomass into bioethanol offers a practical approach to mitigating these environmental impacts while supporting cleaner energy production (Santa-Maria et al., 2013).

According to Dhas et al. (2025), red banana waste (*Musa acuminata*) was used as a substrate for bioethanol production using *Saccharomyces cerevisiae*. The bioethanol yield obtained was 35.9 ± 0.51 g/L, which was 11% higher than the yield under non-optimized conditions.

Santis-Espinosa et al., (2025) found that pretreatment of banana residues significantly increased bioethanol production, with acid hydrolysis yielding 55.18 g/L, sterilization 64.26 g/L, and pasteurization 34.11 g/L, showing that pretreatment improves sugar availability for fermentation). Uljanah et al., (2024) reported that banana pseudostem waste (*Musa balbisiana*) produced 41.5% (v/v) ethanol using 12% yeast over 13 days of fermentation, which was higher than yields from lower yeast concentrations and shorter fermentation times.

Alonso-Gómez et al., (2019) used whole unripe plantain (*Musa paradisiaca* L.) as raw material for bioethanol production. After 32 h of fermentation, the whole plantain produced 5.86 % of bioethanol, while the plantain pulp yielded 4.55 % of bioethanol.

Considerable research has been conducted on bioethanol production from different parts of the banana plant. However, there is a noticeable lack of publications specifically focused on bioethanol production from the peels of *Musa paradisiaca* using *Saccharomyces cerevisiae*. This presents a significant

opportunity to contribute novel findings in this area by utilizing underexplored biomass to meet energy demands. The objectives of the study were to identify the most underutilized parts of *Musa paradisiaca* that yield a higher quantity of bioalcohol and to optimize the culture conditions and media compositions to maximize bioalcohol output.

MATERIALS AND METHODS

Chemicals and culture media

All the chemicals used in this study were obtained from standard sources and were of analytical grade. Hydrolysis was carried out using 1 M H_2SO_4. The basal medium contained 4 g/L yeast extract, 8 g/L KH_2PO_4, 4 g/L $(NH_4)_2SO_4$, 2 g/L peptone, and 4 g/L $MgSO_4.7H_2O$. After autoclaving, 5 g of *Saccharomyces cerevisiae* (commercial yeast, 50 g/L) was added to the conical flask containing the media.

Collection of samples

Musa paradisiaca leaves, bunch stalks, roots, peels, and pseudostems were collected from several sites in the Jaffna district. The samples were packed in polythene and brought to the laboratory.

Inoculum preparation

Saccharomyces cerevisiae (Baker's yeast) was purchased from Cargill's Food City, Jaffna, Sri Lanka. *Saccharomyces cerevisiae* was cultured in 100 ml of sterile glucose (50 g/L) and sucrose (50 g/L) solution by inoculating yeast strains (50 g/L). The culture was shaken at 100 rpm at room temperature for 18 h.

Substrate preparation

Leaves from *Musa paradisiaca* were collected, and the midribs removed. After cleaning under running water, the leaves were air dried. Thereafter, they were oven-dried at 60 ˚C until reaching a constant weight. Finally, the dried leaves were ground into a fine powder and stored in plastic bottles. Bunch stalks, roots, peels, and pseudostems of *Musa paradisiaca* were collected separately, thoroughly cleaned with clean running tap water, sliced into small pieces using a sharp knife, and air dried. All the samples were oven dried at 60 ˚C to a constant weight. Finally they were ground thoroughly with a grinder and stored in plastic bottles.

Production of bioalcohol and alcohol measurement

Physical pre-treatment

After cleaning with tap water, the materials (leaves, bunch stalks, roots, peels, and pseudostems) were chopped into small pieces, air dried, and then oven dried at 60 ˚C until their weight remained constant. The materials were then ground thoroughly in a grinder and stored in bottles.

Pre-treatment via autoclaving

Each sample, weighing thirty grams, was placed in separate 500 mL conical flasks with 100 mL of distilled water. Flasks were sealed air-tight with cotton wool. The samples were then autoclaved for 15 minutes at 121˚C and 1 atm of pressure and left to cool.

Acid hydrolysis

Samples were acid hydrolysed with 100 mL of 1 M H_2SO_4 and autoclaved at 121˚C, 1 atm pressure for 15 minutes. The mixture was cooled to room temperature, filtered through muslin cloth, and the filtrates were centrifuged for 15 minutes at 8000 rpm. The supernatants were collected and neutralized with 4 M NaOH.

Fermentation process

The fermentation medium (75 mL) containing 4 g/L yeast extract, 8 g/L KH_2PO_4, 4 g/L $(NH_4)_2SO_4$, 2 g/L peptone, and 4 g/L $MgSO_4.7H_2O$ was added with 300 mL of neutralized solution in separate conical flasks. The mixture was then autoclaved at 121˚ C and 1 atm pressure for 15 minutes and the solutions were left to cool. The solutions were inoculated aseptically with 10% of an 18 h old culture of *Saccharomyces cerevisiae* inoculum. The mixture was allowed to ferment for 24 h at room temperature while being incubated at 100 rpm in an orbital shaker. For fermentation, the conical flasks were firmly sealed with cotton plugs to create an anaerobic environment.

Measuring alcohol percentage

From the fermented samples, 50 mL were transferred to a falcon tube and centrifuged at full speed (3000 rpm). The supernatant was carefully collected, and the pellet discarded. The ebulliometer method was then used to determine the alcohol percentage of the supernatant (Gnanasegaram et al., 2024). The five samples taken

from *Musa paradisiaca* underwent the same process as before.

Determination of reducing sugar

The reducing sugar content was measured using the 3,5-dinitrosalicylic acid (DNS) method. Standard glucose solutions were prepared by diluting different volumes (0.2–1.0 mL) of a stock glucose solution (1.0 g/L) into labeled test tubes, with the total volume adjusted to 1.0 mL using distilled water. Each tube was then treated with 1.0 mL of DNS reagent and heated in a boiling water bath for 5 minutes. After cooling, 10.0 mL of distilled water was added to each tube, and the absorbance was measured spectrophotometrically at 550 nm with a reagent blank serving as the reference. The reagent blank was prepared by using 1.0 mL of distilled water instead of the standard glucose solution. The glucose concentrations of unknown samples were measured by comparing them to the standard glucose curve after they were treated similarly (Christy et al., 2023).

Distillation and gas chromatography

Absolute ethanol (≥99.9%), methanol (≥99.9%), 3-methyl-1-butanol (≥99%), and 2-methyl-1-propanol (≥99%) standards were obtained from Sigma-Aldrich (USA). High purity helium (99.999%) was used as the carrier gas throughout the chromatographic analysis.

After fermentation was completed, the broth was initially filtered through muslin cloth followed by Whatman No. 1 filter paper to remove any residual solids. The clear filtrate was then distilled fractionally using a conventional laboratory apparatus consisting of a 500 mL round bottom flask fitted with a fractionating column, thermometer, Liebig condenser, and a receiving flask. Distillation was conducted using a heating mantle, and the fraction corresponding to 78–85 °C was collected. The collected distillate was stored in airtight amber bottles at 4 °C for further analysis.

The chemical composition of the distilled samples was determined using a Gas Chromatography–Mass Spectrometry (GC–MS) system (Agilent 7890B GC coupled with 5977A MSD, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium served as the carrier gas, maintained at a constant flow rate of 1.0 mL min^{-1}. A 1 µL sample was injected in split mode (10:1) with the injector temperature adjusted to 250 °C. The oven temperature was initially set at 40 °C for 3 min, then increased at 10 °C min^{-1} to 200 °C, and held for 5 min. Mass spectrometric detection was performed using electron impact ionization at 70 eV, with an ion source temperature of 230 °C and a scan range of m/z 30–300. Compounds were identified by comparing their mass spectra with the NIST library database and confirmed by retention times of authentic standards. The relative percentage composition was calculated based on peak area normalization.

Optimization of conditions for bioalcohol production

Selection of suitable substrate

Leaves, bunch stalks, roots, peels, and pseudostems of *Musa paradisiaca* were used as substrates which underwent pre-treatment, acid hydrolysis and fermentation. The alcohol percentage produced from each substrate was determined and the substrate with the highest percentage of alcohol was selected for further optimization studies.

Optimization of time

Thirty grams of banana peel sample, after pre-treatment and acid hydrolysis, was fermented for 50 h on an orbital shaker at 100 rpm, room temperature. The alcohol percentage was measured every 10 h, and the time with the highest alcohol content was selected for further analysis.

Optimization of different hydrolysis agents

The banana peel sample was hydrolyzed using different acids (1 M H_2SO_4, HCl, and HNO_3) and alkalis (1 M NaOH and KOH), and then autoclaved. The mixture was fermented with *Saccharomyces cerevisiae* for 40 h and the alcohol percentage was determined. The hydrolysis agent, that produced the most alcohol was chosen for further studies.

Optimization of sulfuric acid concentration in acid hydrolysis

The pre-treated banana peel samples were hydrolysed with different concentrations of sulfuric acid (0.50, 0.75, 1.00, 1.25, and 1.50 M). After adding the resultant mixture to fermentation media containing *Saccharomyces cerevisiae*, it was incubated for 40 h at room temperature at 100 rpm and the alcohol percentage determined. The concentration of sulfuric acid, which produced the highest alcohol yield was chosen for further studies.

Optimization of the amount of yeast inoculum

Thirty grams of banana peel samples were pre-treated with distilled water and then acid hydrolyzed using 0.75 M sulfuric acid. Different amounts of yeast inoculum (25, 50, 75, 100, 125, and 150 g/L) were added to conical flasks containing fermentation media. After inoculating aseptically at room temperature, it was left to ferment for 40 h at room temperature in an orbital shaker at 100 rpm and the alcohol percentage was determined. The yeast inoculum concentration that produced the highest alcohol percentage was selected for further studies.

Optimization of the amount of substrate

Different quantities of banana peel samples (15, 30, 45, 60, 75, 90 g /100 mL) were used as substrate to produce alcohol by fermentation after pre-treatment with 0.75 M sulfuric acid for hydrolysis. The fermentation process was carried out using a yeast inoculum concentration of 100 g/L for 40 h and the alcohol percentage was determined.

Statistical analysis

Each experiment was conducted in triplicate, and the average results were displayed graphically. Minitab 17.0 was used for statistical analysis. Tukey's multiple comparison test was used to identify significant differences, at a significance level of $p < 0.05$, after analyzing the data using one-way ANOVA.

RESULTS AND DISCUSSION

Effect of different substrates on bioalcohol production

Various parts of the *Musa paradisiaca* plant such as leaves, bunch stalk, roots, peels, and pseudostem were used as substrates for bioalcohol production. The alcohol percentage was measured under non-optimized conditions using a 24 h fermentation period, 1 M sulfuric acid hydrolysis, 50 g/L yeast inoculum, and a substrate concentration of 30 g/100 mL. *Musa paradisiaca* peels yielded a significantly higher quantity of alcohol (0.2%) compared to other underutilized parts of *Musa paradisiaca* (root 0.03%, pseudostem 0.06%, leaf 0.1%, bunch stalk 0.06%) after 24 h of fermentation. The reducing sugar content also followed the same trend. Banana peels contain considerable amounts of carbohydrates, primarily in the form of fermentable sugars, which are crucial for bioalcohol production. Alcohol production was notably lower in the roots, leaves, bunch stalk, and pseudo-stem due to the absence of sufficient fermentable substances (Figure 1). Therefore, the peels of *Musa paradisiaca* were selected as a carbon substrate for further optimization.

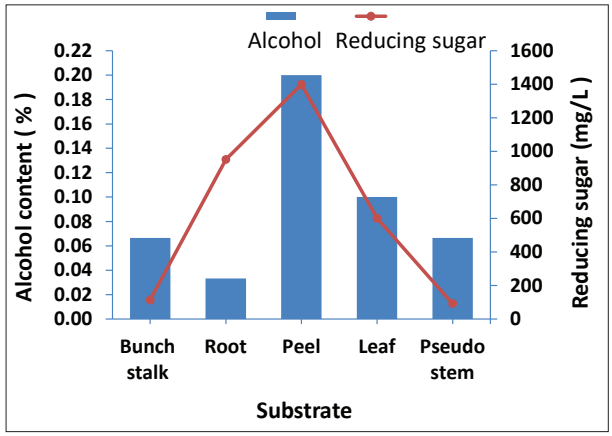

Figure 1: Effect of different plant parts of *Musa paradisiaca* as substrates on bioalcohol production using *Saccharomyces cerevisiae*.

Ilangarathna et al., (2022) used various coconut plant parts, such as young coconut fiber, coconut husk fiber, dried coconut leaves, green coconut leaves, and roots of coconut as carbon substrates for bioethanol production. Among these substrates, green coconut leaves produced the highest quantity of bioethanol compared to others.

Zainab et al., (2022) reported that among the three substrates utilized for bioethanol production - sugarcane bagasse, rice husk, and corn cob - the highest quantity of bioethanol was produced by sugarcane bagasse. Lignocellulosic biomass presents a viable alternative for producing bioethanol, being widely available, cost-effective, and not competing with food or feed crops (Bušić et al., 2018). The low lignin content in banana peels enables easier break down during the hydrolysis and pre-treatment stages. Less lignin indicates that the sugars can be released more efficiently because it may hinder cellulose's accessibility (Hamzah et al., 2019).

Effect of fermentation time

With an increasing fermentation time from 10 to 50 h, the maximum alcohol yield was observed at 40 h (Figure 2), with the yield increasing from 0.2% to 0.53% (v/v). Therefore, 40 h was determined to be the optimum fermentation time and chosen for the subsequent experiments.

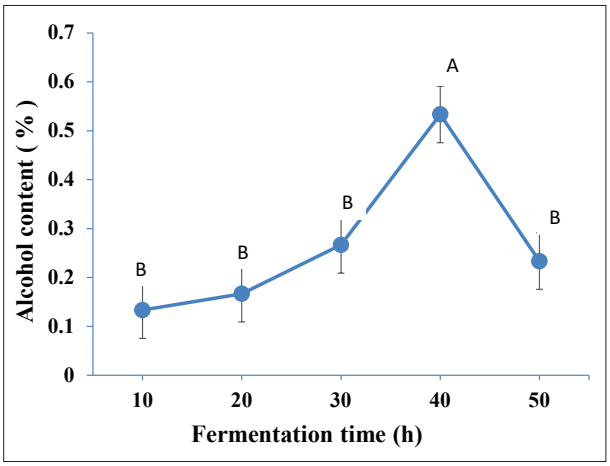

Figure 2: Effect of fermentation time on bioalcohol production from peels of *Musa paradisiaca* using *Saccharomyces cerevisiae.* Significant differences between mean values are indicated by different letters (A, B).

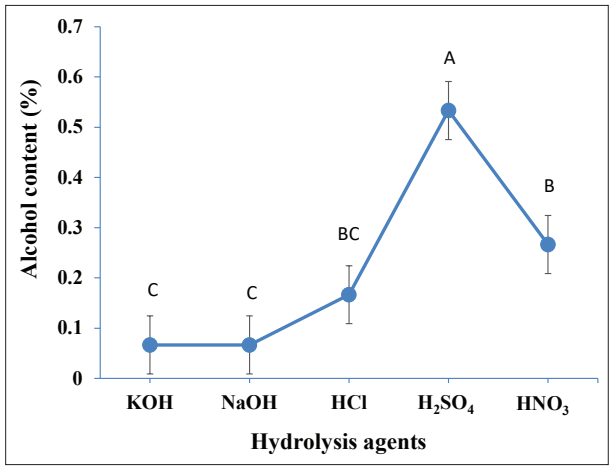

Figure 3: Effect of different hydrolysis agents on bioalcohol production from peels of *Musa paradisiaca* using *Saccharomyces cerevisiae*. Significant differences between mean values are indicated by different letters (A, B, C).

After 30 h of fermentation, a significantly higher bioethanol yield of 10.8% was obtained, which is 1.56 times greater than the yield from non-optimized conditions, using *Saccharomyces cerevisiae* on paddy husk of *Dahanala Red Naadu* (Christy et al., 2024). On the 4th day of fermentation, significantly higher quantities of bioethanol were produced from the whole plant of *Parthenium hysterophorus* using *Saccharomyces cerevisiae* (Gnanasegaram et al., 2024). The length of fermentation has an impact on yeast cell development. Furthermore, yeast cells expend energy during the first phase of fermentation to adjust to the growing environment. However, if the fermentation process is too long, the system's increased bioethanol concentrations could be toxic to the fermenting cells (Christy et al., 2023).

Effect of different hydrolysis agents

After 40 h of fermentation with *Saccharomyces cerevisiae*, hydrolysis of *Musa paradisiaca* peel using 1 M sulfuric acid produced an alcohol yield of 0.53% (v/v), which was significantly higher than the yields obtained with other acids tested (1 M nitric acid, and 1 M hydrochloric acid) and alkaline solutions (1 M sodium hydroxide and 1 M potassium hydroxide) (Figure 3). Therefore, acid hydrolysis using sulfuric acid was chosen for further studies. El-Tayeb et al., (2012) reported that the highest bioethanol was produced when sugar beet waste was treated with sulfuric acid (5% v/v) compared to hydrolyzing with hydrochloric acid, and phosphoric acid.

Production of bioethanol depends on the selection of the most efficient hydrolysis method for biomass. One important stage that greatly affects the process's overall efficiency is the hydrolysis of lignocellulosic material into fermentable sugars (Binod et al., 2011). The aim of the hydrolysis process is to break down the polysaccharide structures, making them more accessible for conversion into monomers by acids or alkalis (Christy et al., 2023).

The primary benefit of acid hydrolysis is its capacity to penetrate lignin and break down cellulose and hemicellulose polymers into individual sugar molecules without the need for any prior pretreatment of the biomass (Verardi et al., 2012). Since concentrated strong acids such as HCl and H_2SO_4 are effective cellulose hydrolyzers, they do not require additional enzymes thereafter, they are frequently used to treat lignocellulosic materials (Harmsen et al., 2010).

Effect of sulfuric acid concentration

When *Musa paradisiaca* peels were hydrolyzed with 0.75 M sulfuric acid, the alcohol yield increased from 0.53% to 0.63% (v/v), which was significantly higher than the yields obtained with other acid concentrations (Figure 4). Therefore, 0.75 M sulfuric acid was chosen for hydrolysis. The concentration of sulfuric acid is important to the hydrolysis process to produce bioalcohol from *Musa paradisiaca* peels. In this study, the production of bioalcohol increased significantly as the concentration of

sulfuric acid increased and reached a maximum value; thereafter, the production of bioalcohol was reduced by further increases in sulfuric acid concentration. The breakdown of sugars by the strong acidity may explain the decrease in bioethanol concentration (Kefale et al., 2012).

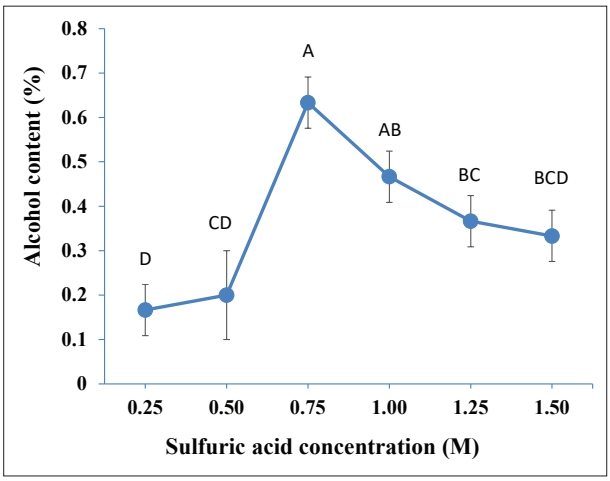

Figure 4: Effect of sulfuric acid concentration on bioalcohol production from peels of *Musa paradisiaca* using *Saccharomyces cerevisiae*. Significant differences between mean values are indicated by different letters (A, B, C, D).

Christy et al., (2023) reported that *C. globularis* produced significantly higher yields of bioethanol and reducing sugar when 0.75 M sulfuric acid was used. In agro-industrial wastes, acid concentrations of 1% sulfuric (v/v) produced a higher quantity of bioethanol than 5% (v/v) (El-Tayeb et al., 2012). High concentrations of H_2SO_4 can lead to browning or charring of the hydrolysate producing unwanted byproducts such as furfural and 5-hydroxymethylfurfural, which are harmful to *Saccharomyces cerevisiae*, hindering fermentation and reducing overall bioethanol production (Mitiku et al., 2020).

Effect of the amount of yeast inoculum

Musa paradisiaca peel produced a significantly higher alcohol yield when the *S. cerevisiae* inoculum concentration was 100 g/L, with the yield increasing from 0.63% to 0.93% (v/v) compared to other inoculum concentrations used (Figure 5). This concentration was selected as the optimum for further studies. Nikolić et al., (2009) tested three different inoculum concentrations (2%, 10%, and 20% (w/v)) using corn meal hydrolyzates; the highest ethanol yield was produced by an inoculum concentration of 2% (w/v). When the yeast inoculum size was increased to 0.5 g/100 mL, the ethanol yield increased significantly by 1.11 times (from 0.90% to 1.00%) in comparison to the non-optimized control inoculum size (Vivekanandaraja et al., 2021).

The inoculum concentration influences sugar utilization and ethanol production, but has little impact on the final ethanol concentration. Once the biocatalyst reaches a certain concentration, they saturate the system, which can reduce the efficiency of bioethanol production. High inoculum concentrations may saturate the system reducing bioethanol efficiency. Higher inoculum concentrations can lead to nutrient limitations or overcrowding, ultimately decreasing the overall ethanol yield (Laopaiboon *et al*., 2007). The inoculum size was increased by adding more yeast cells to the fermentation flask. Increasing the inoculum size led to the rapid increase in the yield of ethanol due to quick growth and multiplication of the yeast cells in the flask. Most of the substrate, which consisted of fermentable sugars, was quickly consumed and transformed into ethanol when additional yeast cells were added. However, yield of ethanol began to decline with further increases in inoculum size, which competed for the limited nutrients in the reactor reducing the multiplication of yeast cells and producing ethanol (Elizabeth et al., 2018).

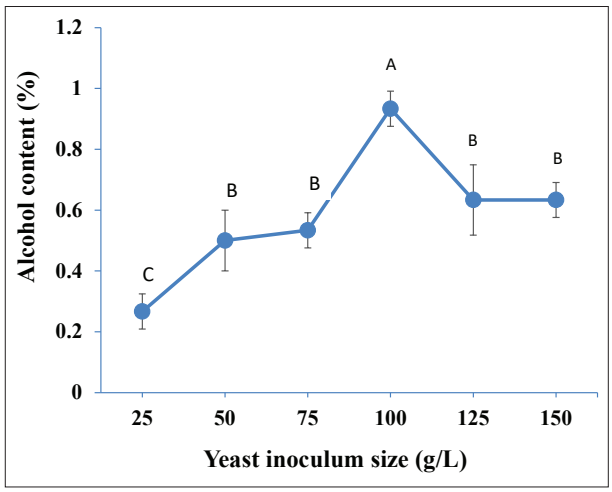

Figure 5: Effect of yeast inoculum size on bioalcohol production from peels of *Musa paradisiaca* using *Saccharomyces cerevisiae*. Significant differences between mean values are indicated by different letters (A, B, C).

Effect of amount of substrate

When evaluating different concentrations of *Musa paradisiaca* peel substrate, the highest alcohol yield was achieved at 75 g/100 mL after 40 h of fermentation, with the yield increasing from 0.93% to 1.63% (v/v). When the concentration of peel ranges from 15 g/100 mL to 75 g/100 mL, the yield of bioalcohol increases exponentially, but it takes longer for the yeast cells to adjust to the medium. However, at concentrations exceeding 75 g/100 mL, a slight decline in alcohol yield was observed (Figure 6). Therefore, 75 g/100 mL of peel substrate was selected as the optimized amount and used for further studies.

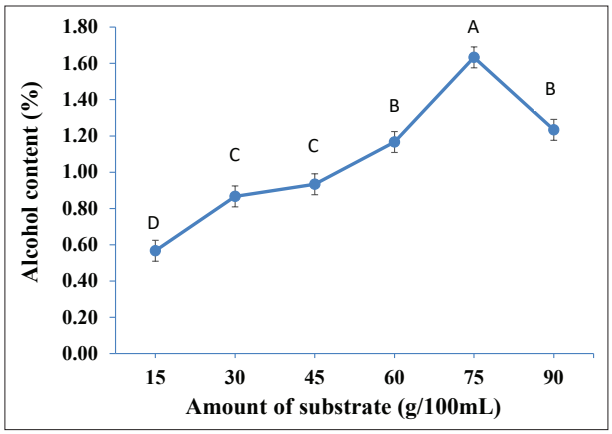

Figure 6: Effect of the amount of substrate on bioalcohol production from peels of *Musa paradisiaca* using *Saccharomyces cerevisiae*. Significant differences between mean values are indicated by different letters (A, B, C, D).

Using oil palm empty fruit bunches as the substrate, Mardawati et al., (2019) determined the production of bioethanol at different concentrations (3%, 6%, 9%, 12%, and 15%); after three h of fermentation, the maximum ethanol concentration was 0.25 g/L, with the highest ethanol yield at 9% concentration. Using three concentrations of cassava peel (10%, 15%, and 20% w/v) as a substrate Abdullah et al., (2024), found 15% concentration yielded the highest bioethanol, with a maximum concentration of 6.172 g/L. Since there is more raw material available for fermentation, increasing the amount of substrate can result in higher ethanol yields but may also cause substrate inhibition. High substrate concentrations may hinder yeast cell activity, a phenomenon known as substrate inhibition. This may result in less ethanol being obtained from fermentable sugars, which would lower the overall quantity of bioethanol produced during fermentation (Tenkolu et al., 2022).

Osmotic stress, which negatively affects yeast cells and reduces their growth and metabolic activity, is the reason for this decrease in ethanol yields (Chang et al., 2018). After the optimization process, the *Musa paradisiaca* peel substrate produced 8.15 times more alcohol than non-optimized conditions (increasing from 0.2 % to 1.63 %).

Distillation and GC analysis for ethanol purity assessment

The GC results for alcohol obtained from *Musa paradisiaca* peel substrate are shown in Figure 7. This showed that the most dominant compound in the sample was ethanol (89.6%) followed by 3-methyl-1-butanol (6.4%), and the least dominant compound was 2-methyl-1-propanol (3.8%). Methanol was found in trace amounts (0.2%). Similarly in the GC results for *C. globularis*, ethanol was the dominant compound (90.1%), followed by 3-methyl-1-butanol (7.3%), with 2-methyl-1-propanol (2.6%) the the least abundant (Christy et al., 2023).

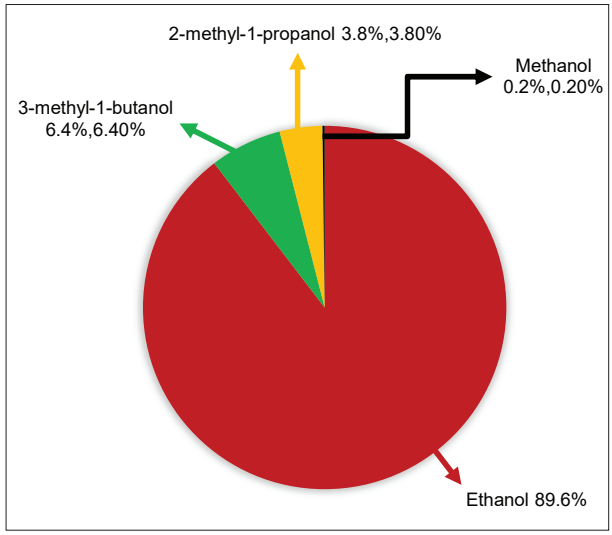

Figure 7: Gas chromatography result for alcohol obtained from *Musa paradisiaca* peel sample.

The high ethanol content and minimal methanol contents indicate efficient fermentation and distillation. The low concentration of higher alcohols and trace impurities suggest improved fuel quality, as excessive fuel alcohols

and methanol can adversely affect combustion efficiency and engine performance (Fini et al., 2021). Therefore, our results demonstrate that *Musa paradisiaca* peel substrate produces bioethanol with comparatively fewer undesirable by-products, supporting its potential as a sustainable and efficient biofuel feedstock.

Table 1: Comparison of optimized ethanol yield (% v/v) obtained in this study with reported yields from banana-based feedstocks.

Study	Substrate	Ethanol yield (% v/v)
This study	*Musa paradisiaca* peels	1.63
Zulnazri et al., 2023	Kepok banana peels (*Musa paradisiaca L.*)	5.04
Bilyartinus et al., 2021	Ambon Banana (*Musa paradisiaca var. sapientum Linn*) Peels	6
Benjamin et al., 2014	Banana peels	6.54
Ogunsuyi et al., 2021	Plantain stem biomass (*Musa paradisiaca L.)*	8.04
Kularathne et al., 2021	Overripe Banana (embul kesel variety)	13.39

Table 1 shows that the ethanol yield obtained in this study (1.63% v/v) is lower than several previously reported studies. The variation in yield may be attributed to differences in substrate type, pretreatment methods, fermentation conditions, and microbial strains used.

CONCLUSION

The peel of *Musa paradisiaca*, an underutilized resource, serves as an efficient substrate for alcohol production when fermented with *Saccharomyces cerevisiae* in liquid media. Optimization of fermentation parameters, including a 40 h fermentation period, sulfuric acid hydrolysis at a concentration of 0.75 M, a yeast inoculum of 100 g/L and 75 g/100 mL of *Musa paradisiaca* peel substrate, resulted in an 8.15-fold increase in alcohol yield, from 0.2% to 1.63% (v/v). Gas chromatography analysis confirmed that the crude alcohol sample contained 89.6% ethanol.

REFERENCES

Abdullah, N. A. E. P. (2024). Kinetics study of bioethanol production from cassava peels waste using *Saccharomyces diastaticus*. *International Journal of Chemical and Biochemical Sciences* (IJCBS), *25*(19), 884-892. https://doi.org/10.62877/106-IJCBS-24-25-19-106

Abdulsalam, A. A. A. S. M., Aliyu, B. B. L., Danillela, U. Y., Ahmad, Z. U., Arul Kumar, B., & Gbadamosi, L. A. (2024). Bioethanol: A sustainable liquid fuel as a substitute to gasoline. *International Journal for Science Technology and Engineering*, *12*(7), 360-371. https://doi.org/10.22214/ijraset.2024.63555

Alonso-Gómez, L. A., Heredia-Olea, E., Serna-Saldivar, S. O., & Bello-Pérez, L. A. (2019). Whole unripe plantain (Musa paradisiaca L.) as raw material for bioethanol production. *Journal of the Science of Food and Agriculture, 99*(13), 5784-5791. https://doi.org/10.1002/jsfa.9847

Assaf, J. C., Mortada, Z., Rezzoug, S. A., Maache-Rezzoug, Z., Debs, E., & Louka, N. (2024). Comparative review on the production and purification of bioethanol from biomass: A focus on corn. *Processes, 12*(5), 1001. https://doi.org/10.3390/pr12051001

Benjamin, C., Singh, P. K., Dipuraj, P. S., Singh, A., Rath, S., Kumar, Y., ... & Peter, J. (2014). Bio-ethanol production from banana peel by simultaneous saccharification and fermentation process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*. *International Journal of Current Microbiology and Applied Sciences, 3*(5), 84-96.

Bilyartinus, G., & Siswanto, A. P. (2021). the effect of bacillus subtilis on bioethanol production from ambon banana (*Musa paradisiaca* var. sapientum Linn) peels by using fermentation process. *Journal of Vocational Studies on Applied Research, 3*(2), 26-30. http://dx.doi.org/10.14710/jvsar.v3i2.11081

Binod, P., Janu, K. U., Sindhu, R., & Pandey, A. (2011). Hydrolysis of Lignocellulosic Biomass for Bioethanol Production. In *Biofuels* (pp. 229–250). Elsevier. https://doi.org/10.1016/B978-0-12-385099-7.00010-3

Broda, M., Yelle, D. J., & Serwańska, K. (2022). Bioethanol production from lignocellulosic biomass - Challenges and solutions. *Molecules, 27*(24), 8717.

https://doi.org/10.3390/molecules27248717

Bušić, A., Marđetko, N., Kundas, S., Morzak, G., Belskaya, H., Ivančić Šantek, M., Komes, D., Novak, S., & Šantek, B. (2018). Bioethanol production from renewable raw materials and its separation and purification: A review. *Food Technology and Biotechnology, 56*(3), 289-311. https://doi.org/10.17113/ftb.56.03.18.5546

Chang, Y.-H., Chang, K.-S., Chen, C.-Y., Hsu, C.-L., Chang, T.-C., & Jang, H.-D. (2018). Enhancement of the efficiency of bioethanol production by Saccharomyces cerevisiae via gradually batch-wise and fed-batch increasing the glucose concentration. *Fermentation, 4*(1), 45. https://doi.org/10.3390/fermentation4020045

Christy, E. J. S. B. A., Branavan, K., & Kapilan, R. (2024). Bioethanol production from rice husk of Dahanala red naadu using Saccharomyces cerevisiae and yield enhancement by optimization of growing conditions. *Vingnanam Journal of Science, 19*(1), 37-43.

Christy, E. J. S. B. A., Kapilan, R., Wickramasinghe, I., & Wijesekara, I. (2023). bioethanol production from Chara globularis using yeast and yield improvement by optimization of conditions. *Ceylon Journal of Science, 52*(2), 219-229. https://doi.org/10.4038/cjs.v52i2.8042

Christy, E.J.S.B.A., Kapilan, R., Wickramasinghe, I. and Wijesekara, I. (2023) Optimization of pre-treatment and culture conditions for bioethanol yield enhancement from Azolla filiculoides substrate using Saccharomyces cerevisiae, *Journal of Dry Zone Agriculture, 9*(1), 1-25. https://doi.org/10.4038/jdza.v9i1.64

Danmaliki, G., Usman, B., Musa, A., & Ahmad, S. (2016). Bioethanol production from banana peels. *IOSR Journal of Environmental Science*, (JESTFT), 10, 56-62. https://doi.org/10.9790/2402-1006025662

Devi, S., Dhaka, A., & Singh, J. (2016). Acid and alkaline hydrolysis technologies for bioethanol production: An overview. *International Journal of Advanced Technology and Engineering Science, 4*(6), 94-106.

Dhas, D. S. D., Thiraviam, A. G. P., Muthuramamoorthy, M., Alodhayb, A., & Arokiyaraj, S. (2025). Bioethanol production from red banana waste via Saccharomyces cerevisiae under optimized conditions. *BioResources*, *20*(4), 10685–10705. https://doi.org/10.15376/biores.20.4.10685-10705

Dimos, K., Paschos, T., Louloudi, A., Kalogiannis, K. G., Lappas A. A, Papayannakos, N., Kekos. D., & Mamma, D. (2019). Effect of various pretreatment methods on bioethanol production from cotton stalks. *Fermentation, 5*(1), 5. https://doi.org/10.3390/fermentation5010005

Edeh, I. (2021). Bioethanol production: An overview. Bioethanol technologies, Chapter 1, 2-3. https://doi.org/10.5772/intechopen.94895

Elizabeth, O., Akwayo, I., Taiwo, O., Obanla, O., Ayoola, A., Ojewumi, E., & Oyeniyi, E. (2018). Bioconversion of sweet potato peel waste to bioethanol using Saccharomyces cerevisiae. *International Journal of Environmental Science and Technology, 8*(3), 46-54.

El-Tayeb, T. S., Abdelhafez, A. A., Ali, S. H., & Ramadan, E. M. (2012). Effect of acid hydrolysis and fungal biotreatment on agro-industrial wastes for obtainment of free sugars for bioethanol production. *Brazilian Journal of Microbiology, 43*(4), 1523-1535. https://doi.org/10.1590/S1517-83822012000400037

Fini, A.T and Fattahi, A. (2021). Bioethanol production from wastes: An experimental evaluating study for Iran. Journal *of Renewable Energy and Environment*, *8*(3), 86-93.

Gnanasegaram, A., & Kapilan, R. (2024). Bioethanol from Parthenium hysterophorus substrate by Saccharomyces cerevisiae. Journal of the University of Ruhuna, 12(1), 28-34. https://doi.org/10.4038/jur.v12i1.8022

Gouthami, Y., Praveen, Gidagiri., G, Bhuvaneshwari., G.S., Lekshmi., & Ms., Shameena. (2024). Bioethanol production from agricultural waste. *Futuristic Trends in Agriculture Engineering and Food Science, 3*, 536-554. https://doi.org/10.58532/V3BCAG15P4CH8

Hackl, M., Jakobi, T., Blom, J., Doppmeier, D., Brinkrolf, K., Szczepanowski, R., Bernhart, S. H., Höner zu Siederdissen, C., Hernandez Bort, J. A., Wieser, M., Kunert, R., Jeffs, S., Hofacker, I. L., Goesmann, A., Pühler, A., Borth, N., & Grillari, J. (2011). Next-generation sequencing of the Chinese hamster ovary microRNA transcriptome: Identification, annotation, and profiling of microRNAs as targets for cellular engineering. *Journal of Biotechnology, 153*(1-2), 62-75. https://doi.org/10.1016/j.jbiotec.2011.02.011

Hamzah, M. A., Alias, A. B., & Ahmad, N. E. (2019). Production of biofuel (bio-ethanol) from fruit waste: Banana peels. *International Journal of Engineering and Advanced Technology, 9*(1), 5897-5901. https://doi.org/10.35940/ijeat.A3024.109119

Harmsen, P., Huijgen, W., Bermudez, L., & Bakker, R. (2010). Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. *Biomass and Bioenergy, 28*(5), 494-506.

Ilangarathna, D. A., & Kapilan, R. (2022). Bioethanol production from coconut fiber wastes using Saccharomyces cerevisiae. *Vingnanam Journal of Science, 17*(1), 9-19 https://doi.org/10.4038/vingnanam.v17i1.4205

Kefale, A., Redi, M., & Asfaw, A. (2012). Potential of bioethanol production and optimization test from agricultural waste: The case of wet coffee processing waste (pulp). I*nternational Journal of Renewable Energy Research, 2*(3), 446-450.

Kularathne, I. W., Gunathilaka, C. A., Ratnaweera, A. C., Kalpage, C. S., Rajapakse, S., & Gamage, P. (2021). Optimization of fermentation process parameters for bioethanol production from Sri Lankan overripe fruits. Engineer: *Journal of the Institution of Engineers, Sri Lanka, 54*(1). http://doi.org/10.4038/engineer.v54i1.7368

Laopaiboon, L., Thanonkeo, P., Jaisil, P., & Laopaiboon, P. (2007). Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by Saccharomyces cerevisiae. *World Journal of Microbiology and Biotechnology, 23*(11):1497-1501. https://doi.org/10.1007/s11274-007-9383-x

Mardawati, E., Putri, A. V., Yuliana, T., Rahimah, S., Nurjanah, S., & Hanidah, I. (2019, February). Effects

of substrate concentration on bioethanol production from oil palm empty fruit bunches with simultaneous saccharification and fermentation (SSF). *IOP Conference Series: Earth and Environmental Science, 230*(1), 012079. https://doi.org/10.1088/1755-1315/230/1/012079

Meenakshisundaram, S., Fayeulle, A., Léonard, E., Ceballos, C., Liu, X., & Pauss, A. (2022). Combined biological and chemical/physicochemical pretreatment methods of lignocellulosic biomass for bioethanol and biomethane energy production: A review. *Applied Microbiology, 2*(4), 716-734. https://doi.org/10.3390/applmicrobiol2040055

Mitiku, A. A., & Hatsa, T. M. (2020). Bioethanol production from decaying fruit peels using Saccharomyces cerevisiae. *International Journal of Current Research and Academic Review, 8*(5), 50-59.

Mushimiyimana, I., & Tallapragada, P. (2016). Bioethanol production from agro wastes by acid hydrolysis and fermentation process. *Journal of Renewable Energy, 75*(6), 383-388.

Nikolić, S., Mojović, L., Rakin, M., Pejin, D., & Nedović, V. (2009). Effect of different fermentation parameters on bioethanol production from corn meal hydrolyzates by free and immobilized cells of Saccharomyces cerevisiae var. ellipsoideus. *Journal of Chemical Technology and Biotechnology*: *84*(4), 497-503. https://doi.org/10.1002/jctb.2068

Ogunsuyi, H. O., & Olawale, C. A. (2021). Evaluation of plantain biomass (Musa paradisiaca L.), as feedstock for bio-ethanol production. *Green and Sustainable Chemistry, 11*(2), 59-71. https://doi.org/10.4236/gsc.2021.112006

Pazmiño-Hernandez, M., Moreira, C. M., & Pullammanappallil, P. (2019). Feasibility assessment of waste banana peduncle as feedstock for biofuel production. *Biofuels, 10*(4), 473-484. https://doi.org/10.1080/17597269.2017.1323321

Santa-Maria, M., Ruiz-Colorado, A. A., Cruz, G., & Jeoh, T. (2013). Assessing the feasibility of biofuel production from lignocellulosic banana waste in rural agricultural communities in Peru and Colombia. *Bioenergy Research, 6*, 1000-1011. https://doi.org/10.1007/s12155-013-9333-4

Santis-Espinosa, L. F., López-Vidaña, E. C., Hernández-Altamirano, R., Grajeda, J. M. S., Aguilar-Aguilar, F. A., & Sebatian, P. J. (2025). Solar Drying and Bioethanol Production From Agro-Industrial Banana (musa paradisiaca l.) Residues. *Revista de Gestão Social e Ambiental*, *19*(1), e010709. https://doi.org/10.24857/rgsa.v19n1-023

Sarkar, N., Ghosh, S. K., Banerjee, S., & Aikat, K. (2012). Bioethanol production from agricultural wastes: An overview. *Renewable Energy, 37*(1), 19-27. https://doi.org/10.1016/j.renene.2011.06.045

Tenkolu, G. A., Kuffi, K. D., & Gindaba, G. T. (2024). Optimization of fermentation condition in bioethanol production from waste potato and product characterization. *Biomass Conversion and Biorefinery, 14*(4), 5205-5223. https://doi.org/10.1007/s13399-022-02974-4

Uljanah, D., Melwita, E., & Novia, N. (2024). Bioethanol Fermentation from Banana Pseudostems Waste (*Musa balbisiana*) Pretreated with Potassium Hydroxide Microwave. *Journal of Ecological Engineering*, *25*(9), 1–13. https://doi.org/10.12911/22998993/190691

Verardi, A., De Bari, I., Ricca, E., & Calabrò, V. (2012). Hydrolysis of lignocellulosic biomass: current status of processes and technologies and future perspectives. Bioethanol, Rijeka InTech, 95-122. https://doi.org/10.5772/23987

Vivekanandaraja, R., & Kapilan, R. (2021). Production and optimization of bioethanol from over ripen sour banana fruit wastes (Musa sapientum) using Saccharomyces cerevisiae. *Journal of dry zone agriculture, 7*(2), 53-76. https://doi.org/10.4038/jdza.v7i2.28

Wandscher Busanello, F., Sérgi Gomes, M. C., Paschoal, S. M., Rodrigues da Silva Baumgärtner, T., Barp, G., & Fiorentin-Ferrari, L. D. (2024). The influence of membrane separation technique in the biodiesel and bioethanol production process: a review. *Biofuels, 15*(7), 903-928. https://doi.org/10.1080/17597269.2023.2294593

Yaverino-Gutiérrez, M. A., Wong, A. Y. C. H., Ibarra-Muñoz, L. A., Chávez, A. C. F., Sosa-Martínez, J. D., Tagle-Pedroza, A. S., & Balagurusamy, N. (2024). Perspectives and progress in bioethanol processing and social economic impacts. *Sustainability, 16*(2), 608-638 https://doi.org/10.3390/su16020608

Zainab, I. S. G., Adiya, S. S., Adamu, M. A., Ibrahim, E. O., & Jamal, D. I. (2022). Comparative study of bioethanol produced from different agro-industrial biomass residues. Earthline Journal of *Chemical Sciences, 7*(2), 143-152. https://doi.org/10.34198/ejcs.7222.143152

Zulnazri, Z., Maulana, A., Muarif, A., Sylvia, N., & Dewi, R. (2023). Production of Bioethanol from Banana Peel (Musa paradisiaca l.) Using Sulfuric Acid Catalyst. *Jurnal Teknologi Kimia Unimal*, *12*(1), 97-111. https://doi.org/10.29103/jtku.v12i1.11654