

“Valorization of Feedstock for Renewable Bioethanol Production”

Ms. Gayatri Madekar

Ms. Mayuri Salve

Ms. Sadia Shaikh

Ms. Sakshi Deshmane

Ms. Shubhangi Shirsath

Ms. Vaishakhi More

-: Under the guidance of: -

Ms. Jyoti Galande

Assistant professor

Department of Biotechnology

Shivchhatrapati College, Chhatrapati Sambhajinagar

SUMMARY

The present study was carried out to produce bioethanol from lignocellulosic biomass using three locally available waste materials: cassava peels, switchgrass, and sawdust. With the increasing demand for renewable and environmentally friendly energy sources, bioethanol has emerged as a promising alternative to fossil fuels due to its clean-burning properties and reduced carbon emissions. Agricultural residues and industrial waste biomass are rich in cellulose, hemicellulose, and starch components, which can be hydrolysed into fermentable sugars and subsequently converted into ethanol through microbial fermentation. Utilizing such waste feedstocks not only supports biofuel production but also reduces biomass disposal issues, helps mitigate pollution, and promotes sustainable waste-to-energy conversion.

Cassava peels were collected from local food processing units, switchgrass from agricultural land, and sawdust from a nearby sawmill. Each biomass sample was washed to remove impurities, dried at controlled temperature, and ground to fine powder to increase surface area for hydrolysis. Pretreatment was performed using dilute sulfuric acid to disrupt the complex lignocellulosic matrix and release reducing sugars. Hydrolysates were neutralized to a suitable pH and inoculated with activated *Saccharomyces cerevisiae* yeast for fermentation. Fermentation was carried out under anaerobic conditions at 40 °C for 18 hours, enabling conversion of simple sugars into ethanol.

Upon completion of fermentation, the fermented broth was distilled at 78 °C to collect purified ethanol. The distillate was subjected to qualitative analysis to confirm ethanol production. The flammability test showed a clean blue flame indicating the presence of ethanol. The iodoform test produced a pale-yellow precipitate confirming the $\text{CH}_3\text{CH}(\text{OH})\text{--}$ group characteristic of ethanol, while the chromic acid test resulted in a visible colour change from orange to green, verifying successful oxidation of a primary alcohol. These positive tests confirmed that ethanol was successfully produced from cassava peels, switchgrass, and sawdust.

This study supports the feasibility and potential of converting lignocellulosic and starchy biomass waste into renewable bioethanol through pretreatment, fermentation, and distillation. Bioethanol derived from waste biomass holds

considerable advantages such as lower emissions, biodegradability, and compatibility with existing fuel infrastructure. Moreover, the approach supports circular economy models by transforming waste materials into valuable commercial energy products. The conversion of waste into bioethanol contributes to environmental conservation by reducing open biomass burning, lowering greenhouse gas emissions, and providing an economically viable renewable fuel source.

Beyond energy applications, bioethanol can be used as an industrial solvent, disinfectant, and fuel additive to reduce vehicle emissions when blended with petrol. Production of bioethanol from locally available biomass has the potential to create employment opportunities, stimulate rural development, and reduce dependency on imported petroleum. This aligns with national sustainable energy policies promoting biofuel development and waste valorization.

Although the present study successfully demonstrated ethanol production at laboratory scale, future research must focus on improving pretreatment efficiency, optimizing fermentation conditions, enhancing microbial tolerance, and increasing ethanol yield. Advanced pretreatment techniques such as enzymatic hydrolysis, steam explosion, and alkaline treatment could potentially improve sugar recovery. Future studies may also explore genetically engineered or stress-tolerant microbial strains for co-fermentation of pentose and hexose sugars. Integration of advanced separation methods such as membrane filtration or molecular sieves could further improve ethanol recovery.

Scaling up this process to industrial levels will require techno-economic evaluation, process optimization, and integration within biorefinery frameworks where multiple valuable by-products, including lignin, biogas, and organic acids, can be recovered. Such integration increases economic feasibility and reduces waste generation.

Overall, this research establishes that lignocellulosic biomass such as cassava peels, switchgrass, and sawdust are suitable feedstocks for sustainable bioethanol production. The biochemical conversion approach used in this study demonstrated strong potential for replacing traditional fossil fuels and offers a pathway toward achieving renewable energy goals and environmental sustainability. The successful laboratory-scale production of ethanol highlights its importance in addressing global energy challenges and promoting a greener future.

AIMS & OBJECTIVES

- ❖ **Collection of Sample from natural resources (Sawdust, Switch grass, and Cassava) and pre-treatment of Sample.**
- ❖ **Fermentation and collection of bioethanol.**
- ❖ **Purification and physiochemical analysis of bioethanol.**
- ❖ **Application of bioethanol.**

INTRODUCTION

The growing global demand for energy, combined with increasing environmental degradation and the depletion of fossil fuels, has prompted a global shift toward alternative energy sources. Fossil fuels, which currently dominate the global energy landscape, contribute significantly to greenhouse gas emissions and climate change. As a result, the search for renewable, sustainable, and ecofriendly energy alternatives has gained considerable momentum. Among these, biofuels have appeared as a promising solution, offering the potential to meet energy needs while reducing environmental impacts. (Balat, M., Balat, H., & Öz, C. 2008).

One of the most widely used biofuels is bioethanol. Bioethanol is a clean burning; high octane fuel produced primarily through the fermentation of sugars by microorganisms. It is considered a first-and second-generation biofuel, depending on the feedstock used. First-generation bioethanol is derived from sugar-and starch-based crops such as sugarcane and

maize while second-generation bioethanol is produced non-food lignocellulosic biomass like agricultural residues, forestry waste, and industrial by-products. Second-generation feedstocks are more sustainable as they do not compete for food production and are often abundant in waste streams. A notable feedstock gaining attention in second-generation bioethanol production is cassava peel. Cassava (*Manihot esculenta* crantz) is a staple crop widely cultivated in tropical and subtropical regions, particularly in Africa and Asia. Its peels, typically discarded as waste, are rich in carbohydrates and there are four presents a valuable substrate for bioethanol production. The use of cassava peel not only provides low-cost raw material but also offers a sustainable solution for agricultural waste management. (Marszałek & Kamiński 2008).

The bioethanol production process from cassava peels involves several steps. First is pretreatment, which is essential for breaking the complex lignocellulosic structure and enhancing the accessibility of fermentable sugars. Acid hydrolysis, often using sulfuric acid (H_2SO_4), is commonly employed to breakdown starches into simple sugars like glucose. Like cassava, saw dust, a by-product of the timber industry, is another lignocellulosic waste material gaining recognition for bioethanol production. Saw dust is composed primarily of cellulose, hemicellulose, and lignin barrier this process releases simple sugars. Both cassava peel and saw dust offer significant advantages for bioethanol production: they are low-cost, abundantly available, and reduce environmental pollution through waste valorization. There is utilization also aligns with global goals for climate change mitigation, renewable energy promotion, and rural economic development. In addition, crops like switchgrass (*panicum virgatum* L.), a C4 perennial grass, have been explored due to their high biomass productivity, high cellulose contained, and ability to grow on marginal soils with low nitrogen input under rainfed conditions. Switchgrass is a strong candidate for future bioethanol projects, particularly due to its adoptability and yield potential in diverse climates. Among these, cassava peels are an excellent raw material for bioethanol. Cassava is a major crop in tropical countries, and its peels are often thrown away as waste. However, they are rich in carbohydrates and starch, which can be easily converted into ethanol after acid pretreatment and fermentation using yeast like *Saccharomyces cerevisiae*. This process converts simple sugars into ethanol and carbon dioxide. (Kumar et al. 2009).

Sawdust, a waste product from the wood industry, is also a reliable source of fermentable sugars. Although it has high lignin content, which makes the sugars hard to access, chemical pretreatment can break down the structure and release sugars for fermentation.

Another potential feedstock is switchgrass (*Panicum virgatum*), a fast-growing grass that can grow in poor soil and requires extraordinarily little fertilizer. It produces high biomass and contains cellulose, which can be converted into ethanol through enzymatic or acid hydrolysis followed by fermentation. Using these materials for bioethanol production is not only cost-effective but also helps with waste management and supports clean energy development. It reduces pollution, supports rural farmers, and contributes to sustainable development goals. Therefore, the use of waste materials such as cassava peels, sawdust, and switchgrass in bioethanol production not only helps reduce environmental pollution but also offers a cost-effective, sustainable, and economically viable solution to global energy problems. It supports waste management, reduces reliance on fossil fuels, and contributes to rural development and climate change mitigation. Switchgrass not only offers an environmentally sustainable route to ethanol production, but also contributes to greenhouse gas mitigation, soil conservation, and carbon sequestration. (McLaughlin & Kszos 2005). According to (Schame et al. 2008), ethanol derived from switchgrass can reduce greenhouse gas emissions by over 90% compared to gasoline on a life cycle basis. Additionally, its perennial nature ensures reduced soil erosion and better land-use efficiency. Despite its advantages, the commercial-scale implementation of bioethanol production from switchgrass faces several challenges. These include high enzyme costs, difficulties in lignin removal, and the need for optimized pretreatment technologies. Continuous research and development, supported by policy incentives and technological innovation, are critical to overcoming these barriers and making bioethanol from switchgrass economically viable. Sawdust, a waste product from the wood industry, is also a reliable source of fermentable sugars. Although it has high lignin content, which makes the sugars hard to access, chemical pretreatment can break down the structure and release sugars for fermentation. Advanced methods, including ionic liquid pretreatment and genetically modified yeast strains, have shown promising results in enhancing ethanol yields from sawdust. Using these materials for bioethanol production is not only cost-effective but also helps with waste management and supports clean energy development. It reduces

pollution, supports rural farmers, and contributes to sustainable development goals. Therefore, the use of waste materials such as cassava peels, sawdust, and switchgrass in bioethanol production not only helps reduce environmental pollution but also offers a cost-effective, sustainable, and economically viable solution to global energy problems. In conclusion, the production of bioethanol from lignocellulosic waste such as cassava peel, saw dust, and dedicated energy crops like Switchgrass represents a sustainable, economical, and environmentally sound approach to energy generation. The integration of efficient pretreatment and fermentation processes ensures high ethanol yield, waste reduction, and support for green technologies. This strategy not only contributes to global energy security but also supports environmental conservation and rural development in biomass-rich regions.

REVIEW OF LITERATURE

Bioethanol has gained enormous global attention due to the declining reserves of fossil fuels and the increasing need for clean and renewable energy. According to Demarrias (2007), bioethanol is a sustainable fuel that can be produced from a variety of biomass sources including sugar-based, starch-based and lignocellulosic feedstocks.

The author emphasized that bioethanol not only helps in reducing air pollution but also contributes to rural economic development by generating employment opportunities in agriculture and industrial sectors.

Moreover, the blending of bioethanol with gasoline increases the octane number and improves engine performance. Demirbas further highlighted that countries with abundant agricultural resources can achieve energy security by adopting bioethanol technology.

Therefore, research on improved feedstocks and production methods is essential for enhancing bioethanol commercialization.

Sriroth et al. (2000) stated that cassava is an important starchy crop suitable for bioethanol generation due to its high starch content, rapid growth and adaptability to different climatic conditions.

Cassava requires low agricultural inputs and can tolerate drought conditions, making it more efficient than many other starch crops. The authors also mentioned that cassava-based ethanol production reduces production cost when compared to crops like corn or wheat.

Cassava peel and pulp waste generated during starch extraction can be effectively utilized to produce ethanol, thereby supporting waste-to-energy approaches. Additionally, research efforts have been focused on improving enzymatic hydrolysis efficiency of cassava starch to enhance fermentable sugar release.

Hence, cassava stands as a reliable and low-cost substrate for large-scale ethanol manufacturing.

Sanderson (2011) described switchgrass as a second-generation biofuel crop with high biomass yield potential. Being a perennial crop, switchgrass does not require frequent replanting and supports year-round biomass supply.

It contains around 40–50% cellulose and 30% hemicellulose which are major sugar polymers for ethanol formation. Sanderson also pointed out that switchgrass cultivation improves soil fertility, prevents soil erosion and increases biodiversity.

Another significant aspect is that switchgrass cultivation can convert degraded land into productive energy plantations without affecting food crop cultivation. Due to these advantages, switchgrass has been recognized as an ideal lignocellulosic feedstock for future bioenergy programs.

Chandel et al. (2017) reported that simultaneous saccharification and fermentation (SSF) has several operational benefits over other technologies. SSF reduces sugar accumulation in the medium, prevents product inhibition and enhances ethanol productivity.

The study highlighted that SSF can be combined with thermotolerant yeast strains to achieve higher fermentation rates. Cost reduction in industrial operations is becoming possible due to the requirement of fewer reactors and shorter processing time. SSF is therefore a promising technology for industrial commercialization of lignocellulosic ethanol.

Huang et al. (2009) explained that sawdust, wood chips and forestry residues are excellent lignocellulosic materials produced in massive quantities globally. Their utilization for bioethanol production helps reduce solid waste accumulation and prevents environmental degradation caused by open burning or landfill disposal.

The authors highlighted that pretreatment techniques are essential to break the complex structure of lignin and improve the release of cellulose and hemicellulose during hydrolysis. Furthermore, integrating advanced enzymatic cocktails has shown improved sugar yields from sawdust biomass. Researchers are also exploring microbial strains that can efficiently ferment sugars derived from sawdust hydrolysates.

Limayem and Ricke (2012) focused on the barriers limiting large-scale production of lignocellulosic ethanol. The major challenges include high pretreatment cost, formation of inhibitory compounds and limited microbial fermentation efficiency.

Traditional yeast strains such as *Saccharomyces cerevisiae* can ferment only hexose sugars, leaving pentose sugars unutilized. Therefore, metabolic engineering and development of genetically modified organisms (GMOs) such as *Zymomonas mobilis* and *Pichia stipitis* are crucial for efficient utilization of both sugar types. The authors recommended further improvements in pretreatment and detoxification strategies to make industrial production sustainable and cost-effective.

In recent studies, researchers have focused on valorization of agro-industrial wastes such as sugarcane bagasse, rice straw, wheat straw, fruit peels and brewery waste to produce bioethanol. These residues are rich in carbohydrates and available in massive quantities at extremely low cost.

Valorization supports the circular economy concept by converting waste materials into valuable products and minimizing ecological impacts. Improvements in enzyme engineering and pretreatment methods like steam explosion, ionic liquid pretreatment and biological delignification have significantly enhanced ethanol yields from lignocellulosic materials.

Additionally, the integration of downstream processes such as membrane separation and distillation has improved ethanol purification and recovery efficiency.

From the above studies, various agricultural and lignocellulosic feedstocks such as cassava, switchgrass, sawdust and other biomass residues possess significant potential for the production of renewable bioethanol. Researchers have also developed advanced technologies such as pretreatment and SSF to improve conversion efficiency.

However, challenges like high processing cost, inhibitory compounds and limited microbial fermentation efficiency remain. Therefore, further research is needed to explore low-cost, eco-friendly feedstocks and efficient processing methods to make bioethanol production more sustainable and economically viable.

MATERIAL AND METHODS

1. Sample collection:

Lignocellulosic biomass samples were collected from different local sources within the Chhatrapati Sambhajnagar region. Sawdust waste was obtained from a nearby timber processing site, cassava peels were collected from a local vegetable market, and switchgrass samples were collected from agricultural fields in Zalta village. The collected materials were transported to the laboratory in sterile polyethylene bags and stored at room temperature until further processing.

2. Pretreatment:

• Cassava Peels:

Cassava peels were washed thoroughly under running tap water to remove soil and impurities. The cleaned peels were sun-dried for 48 hours, followed by oven-drying at 60 °C for 24 hours to remove residual moisture. Dried peels were ground using a mechanical grinder to obtain a fine powder and stored in airtight containers.

• Switchgrass and Sawdust:

Switchgrass and sawdust samples were washed with distilled water to remove debris and dried at 60 °C for 24 hours. The dried materials were chopped and milled using a pulverizer to produce small particle-sized biomass, ensuring increased surface area for subsequent hydrolysis.

3. Acid Hydrolysis:

For hydrolysis, 20 g each of cassava peel powder, sawdust, and switchgrass were measured separately. Acid treatment was performed by adding 0.5 M H₂SO₄ in volumes of 100 mL, 100 mL, and 300 mL, respectively. Each mixture was placed in a boiling water bath maintained at 98 °C for 2 hours. After hydrolysis, the samples were sterilized at 121 °C

for 15 minutes in an autoclave, cooled to room temperature, and filtered using Whatman No.1 filter paper. The filtrates (hydrolysates) were collected and stored at 4 °C for subsequent fermentation experiments.

4. Activation of Yeast:

Commercial dry yeast granules were activated in warm sterile water containing 1 g of glucose. The suspension was incubated at 37 °C for 30 minutes to allow cell activation and viability recovery. The pH was adjusted to 4.5 using 1 M HCl. A volume of 2 mL of activated yeast culture was aseptically inoculated into sterile fermentation flasks. The flasks were incubated in a water bath at 40 °C for 6 hours to enrich the yeast population prior to fermentation.

5. Fermentation:

The pretreated and hydrolyzed biomass solutions were inoculated with activated yeast and incubated under anaerobic fermentation conditions. Fermentation was conducted at 40 °C for 18 hours. During this period, the yeast converted the fermentable sugars released from hydrolyzed biomass into ethanol with continuous release of CO₂ gas, indicating active fermentation. Progress of fermentation was monitored by observing gas formation and changes in Odor.

6. Distillation:

After fermentation, the broth was subjected to fractional distillation to recover ethanol. Distillation was performed using a distillation setup maintained at 78 °C, corresponding to the boiling point of ethanol. Ethanol vapors were passed through a water-cooled condenser and collected in a clean receiving flask. The condensed distillate represented purified bioethanol, which was stored in airtight containers and preserved at 4 °C for further analysis.

7. Determination Specific Gravity:

Specific gravity or relative gravity is a dimensionless quantity that is defined as the ratio of the density of a substance to the density of water at a specified temperature and pressure to find the percentage of ethanol.

Procedure:

- 1) A clean and dry specific gravity bottle was weighed, and its empty weight was recorded (**W₁**).
- 2) The bottle was filled with distilled water, and the weight was recorded (**W₂**).
- 3) The bottle was emptied, dried, and then filled with the bioethanol sample. The weight was recorded (**W₃**).

- Specific gravity was calculated using the following formula:

$$\text{Specific gravity} = \frac{\text{Density of Substance}}{\text{Density of Water}} \left[\frac{W_3 - W_1}{W_2 - W_1} \right]$$

where:

W₁=weight of empty bottle

W₂ = weight of bottle filled with water

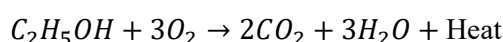
W₃ = weight of bottle filled with bioethanol.

The ethanol percentage was estimated by comparing the calculated specific gravity values with standard specific gravity–ethanol concentration reference data.

❖ Physiochemical Analysis of Bioethanol:

1. Flammability Test:

Ethanol is a flammable organic compound that burns with a characteristic **blue flame** due to its clean combustion. When ignited, ethanol reacts with atmospheric oxygen to produce carbon dioxide and water vapor, releasing heat energy.



Requirements:

- Bioethanol sample (2–3 mL)
- Ceramic evaporating dish / metal spatula
- Spirit lamp / matchstick / lighter

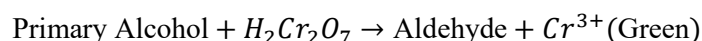
Procedure:

- 1) Place 2–3 mL of bioethanol sample in a clean ceramic dish.
- 2) Ignite the surface gently using a flame source.
- 3) Observe the colour and intensity of the flame.

❖ Quality Analysis Test:

1. Chromic acid test:

Chromic acid ($H_2Cr_2O_7$) acts as a strong oxidizing agent and oxidizes **primary alcohols** to aldehydes. During oxidation, the **orange color** of chromic acid is reduced to **green** due to the formation of Cr^{3+} ions.



Requirements:

- Bioethanol sample (1 mL)
- Chromic acid reagent (Potassium dichromate + conc. H_2SO_4)
- Test tubes
- Pipette

Procedure:

- 1) Add 1 mL of ethanol sample into a clean test tube.
- 2) Add 3–4 drops of chromic acid reagent.
- 3) Shake gently and allow the mixture to stand for 2–3 minutes.
- 4) Observe any color change.

2. Iodoform Test:

The iodoform test detects the presence of a $-\text{CH}_3-\text{CH}(\text{OH})-$ group, characteristic of ethanol. In alkaline conditions, iodine reacts with ethanol to form **iodoform (CHI_3)**, a yellow precipitate with a distinct antiseptic odor.

Ethanol $\rightarrow$ Acetaldehyde $\rightarrow$ CHI_3 (Yellow ppt)

Requirements:

- Bioethanol sample (2 mL)
- Iodine solution (I_2)
- Sodium hydroxide solution (NaOH)
- Water bath
- Test tubes

Procedure:

- 1) Add 2 mL of ethanol sample to a clean test tube.
- 2) Add iodine solution until the solution turns dark brown.
- 3) Add NaOH dropwise until the solution becomes colorless or pale.
- 4) Warm the mixture in a water bath for 2–3 minutes.
- 5) Allow the solution to stand and observe precipitate formation.

❖ Application of Bioethanol:**• Finger Impression Test for Detection of Bioethanol Activity:**

Detect the antimicrobial effect of bioethanol using the finger impression method. Bioethanol has antimicrobial properties. When fingers treated with ethanol are pressed on a nutrient agar plate, fewer bacterial colonies grow compared to untreated fingers.

Requirements:

- Nutrient agar plates (5 plates)
- Bioethanol (70%)
- Sterile cotton
- Marker
- Incubator (37°C)
- Two different persons

Procedure:

1) Label all five nutrient agar plates properly.

2) **Control Plate:**

A finger impression was taken from Person 1 without any ethanol treatment to determine normal microbial load.

3) **Standard Plate:**

Person 1's finger was treated with 70% ethanol (standard disinfectant), allowed air to dry for 30 seconds, and then pressed gently onto the nutrient agar surface.

4) **Sample Plates:**

Different bioethanol samples were applied separately to the fingers of Person 2. After air drying, finger impressions were taken on labelled agar plates.

5) All plates were incubated at **37°C for 24 hours**.

6) After incubation, microbial growth was assessed by observing and comparing the number of bacterial colonies formed on each plate.

7) Following incubation, microbial growth was evaluated by observing colony formation on each plate. The colony count was recorded and compared between control, standard, and bioethanol-treated samples to assess antimicrobial activity.

RESULT AND DISCUSSION

1) Collection of Sample:

Lignocellulosic biomass samples were collected from different local sources within the Chhatrapati Sambhajnagar region. Sawdust waste was obtained from a nearby timber processing site, cassava peels were collected from a local vegetable market, and switchgrass samples were collected from agricultural fields in Zalta village. The collected materials were transported to the laboratory in sterile polyethylene bags and stored at room temperature until further processing.



Switchgrass Sample

Cassava Sample

Saw Dust Sample

Fig. 1: Sample Collection

2) Pretreatment:

• Cassava Peels:

Cassava peels were washed thoroughly under running tap water to remove soil and impurities. The cleaned peels were sun-dried for 48 hours, followed by oven-drying at 60 °C for 24 hours to remove residual moisture. Dried peels were ground using a mechanical grinder to obtain a fine powder and stored in airtight containers.



Fig. 2: Cassava Peel

• Switchgrass and Sawdust:

Switchgrass and sawdust samples were washed with distilled water to remove debris and dried at 60 °C for 24 hours. The dried materials were chopped and milled using a pulverized to produce small particle-sized biomass, ensuring increased surface area for next hydrolysis.



Fig. 3: Switchgrass and Sawdust

3)

Acid Hydrolysis:

Acid Hydrolysis Cassava peel powder (20 g), Sawdust (20 g), and Switchgrass (20 g) were hydrolyzed with 0.5 M H₂SO₄ (100 mL, 100 mL, and 300 mL, respectively). The mixtures were heated in a boiling water bath at 98 °C for 2 h, sterilized at 121 °C for 15 min, cooled, and filtered through Whatman filter paper. The hydrolysates were stored at 4 °C for further use.



Fig.4: Acid Hydrolysis

4) Activation of Yeast:

Yeast granules were inoculated with 1 g of glucose in warm water and incubated at 37 °C. The pH was adjusted to 4.5, and 2 mL of yeast was aseptically added to sterile conical flasks. The flasks were incubated in a boiling water bath at 40 °C for 6 h.

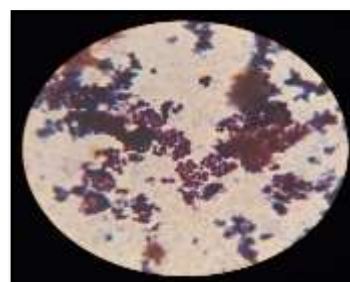


Fig. 5: Yeast Granules

5) Fermentation:

Fermentation The inoculated flasks containing pretreated feedstocks were incubated under controlled conditions to allow yeast-mediated fermentation. During this process, yeast converted fermentable sugars into bioethanol with the release of carbon dioxide. Fermentation was conducted at 40 °C for 18 hours.



Fig. 6: Filtrate Sample

6) Distillation:

The fermented liquid was distilled at 78 °C using a distillation column with water-cooled condensation. Ethanol vapors were condensed and collected in a receiving flask, yielding purified bioethanol.



Fig.7: Distillation

7) Determination of Specific Gravity and Ethanol Percentage:

The specific gravity of fermented bioethanol samples was determined using a specific gravity bottle at room temperature. The values were calculated using the standard formula.

The calculated specific gravity values were used to estimate the percentage of ethanol in each sample.

The results are presented in Table 1.

Sample Name	Specific Gravity	Ethanol (%)
Cassava	0.885	33.92%
Switchgrass	0.925	70.29%
Sawdust	0.990	0.56%

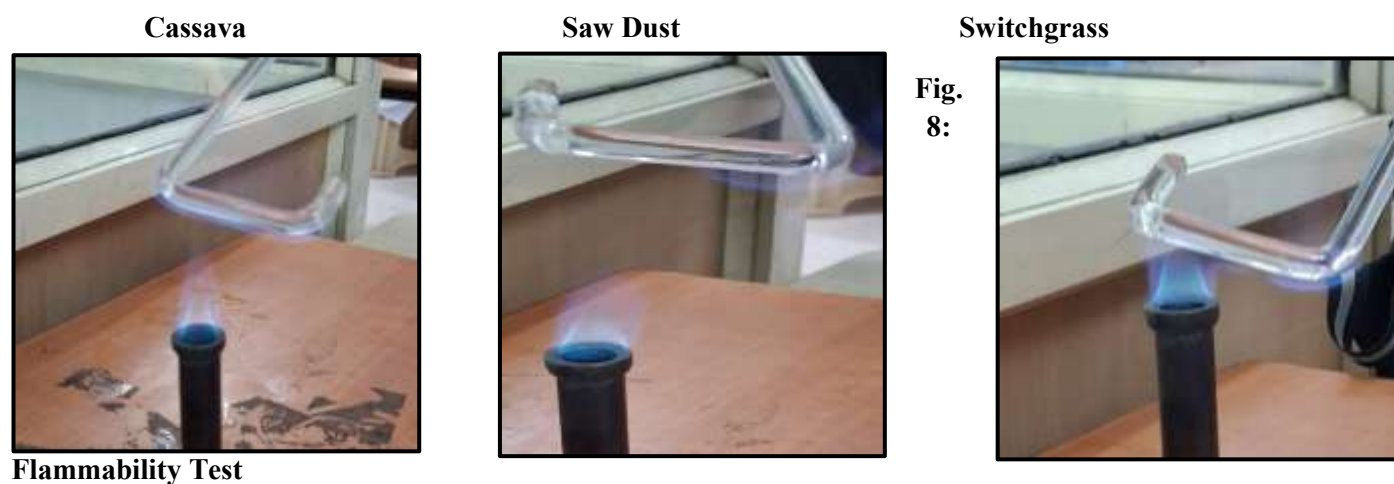
Table 1: Specific Gravity and Percentage of Bioethanol

The results indicate variation in ethanol yield among different substrates. Switchgrass showed the highest ethanol percentage, while cassava and sawdust exhibited the lowest ethanol content.

❖ Physiochemical Analysis of Bioethanol:

1. Flammability Test:

Ethanol is a flammable organic compound that burns with a characteristic **blue flame** due to its clean combustion. When ignited, ethanol reacts with atmospheric oxygen to produce carbon dioxide and water vapor, releasing heat energy.



Flammability Test

❖ Quality Analysis Test:

1. Chromic Acid Test:

Chromic acid ($\text{H}_2\text{Cr}_2\text{O}_7$) acts as a strong oxidizing agent and oxidizes **primary alcohols** to aldehydes. During oxidation, the **orange color** of chromic acid is reduced to **green or blue** due to the formation of Cr^{3+} ions.



Fig. 9: Chromic Acid Test

2. Iodoform Test:

The iodoform test detects the presence of a $-\text{CH}_3-\text{CH}(\text{OH})-$ group, characteristic of ethanol. In alkaline conditions, iodine reacts with ethanol to form **iodoform** (CHI_3), a yellow precipitate with a distinct antiseptic odor.



Cassava Test



Switchgrass Test



Sawdust Test

Fig. 10: Iodoform Test

❖ Application of Bioethanol:

➤ Finger Impression Test for Detection of Bioethanol Activity:

The antimicrobial activity of bioethanol was assessed using the finger impression method on nutrient agar plates. After incubation, the control plate (untreated finger) showed dense microbial growth. In contrast, the bioethanol-treated plates exhibited a marked reduction in colony formation.

The significant decrease in colony count compared to the control indicates the effective antimicrobial potential of bioethanol.



Finger Test Switch Grass



Finger Test Cassava &Sawdust

Fig. 11: Finger Impression Test

Observation:

	Control	Std. Alcohol	Cassava	Sawdust	Switchgrass
No. of colonies	86	07	38	48	18

Table: 2: - Observation of No. of Colonies

The control plate showed the highest microbial count (86 CFU), while standard alcohol showed the lowest (07 CFU). Among the bioethanol samples, switchgrass (18 CFU) exhibited the greatest antimicrobial activity, followed by cassava (38 CFU) and sawdust (48 CFU).

All bioethanol samples demonstrated a reduction in microbial load compared to the control.

DISCUSSION

The present study successfully demonstrated the production of bioethanol from lignocellulosic biomass sources including **sawdust**, **switchgrass**, and **cassava peels** through acid hydrolysis and yeast-mediated fermentation. The results reveal significant differences in ethanol yield among the three substrates, which can be attributed to variations in their chemical composition, particularly cellulose and lignin content.

Among the feedstocks used, **switchgrass produced the highest yield of bioethanol**, indicating that it contains a higher proportion of fermentable carbohydrates suitable for ethanol production. As an herbaceous energy crop rich in cellulose and hemicellulose, switchgrass demonstrates strong potential as a renewable bioenergy source.

Cassava peels produced a moderate quantity of ethanol, which may be attributed to their carbohydrate composition. However, their structural characteristics might limit maximum sugar release under the applied hydrolysis conditions.

The relatively **lower ethanol yield from sawdust** among the tested feedstocks. The high lignin content and rigid woody structure of sawdust may have limited the efficiency of acid hydrolysis, thereby reducing the availability of fermentable sugars for ethanol production.

Fermentation results demonstrated vigorous yeast activity in all samples, confirmed by visible gas release and by qualitative ethanol tests. The **flammability test** confirmed ethanol presence in all samples, producing a characteristic blue flame. Both **chromic acid and iodoform tests** confirmed the presence and purity of ethanol based on expected colour changes and the formation of characteristic precipitate. These observations validated successful conversion of fermentable sugars into ethanol.

Overall, the findings support the utilization of lignocellulosic biomass as an inexpensive and sustainable raw material for renewable energy production, reducing dependence on fossil fuels and contributing to waste management strategies.

CONCLUSION

Bioethanol was successfully produced from cassava peels, switchgrass, and sawdust through acid hydrolysis, yeast fermentation, and distillation. Among the tested biomass sources, **Switchgrass demonstrated the highest ethanol yield**, followed by **cassava peels and sawdust**. Physiochemical and qualitative analyses confirmed the presence and purity of the produced ethanol through flammability, chromic acid, and iodoform tests.

The study concludes that **lignocellulosic biomass represents a promising and renewable feedstock for sustainable bioethanol production**, particularly sawdust, which proved most efficient. Utilizing waste biomass for energy generation can help reduce environmental pollution, improve waste management, and provide an eco-friendly alternative to fossil fuels.

REFERENCES

1. Irene Akinyi Odongo, Elly Tetty Osewe, Sylvia Opiyo. "Production and characterization of Bioethanol from Cassava peels." Volume 17, issue five seer, May 2014.
2. Oyeleke Sb, Dauda Ben, Oyewole Oa, Okaliegbe In, Ojebode T. Production of Bioethanol from Cassava and Sweet Potato Peels. Published Online 2012. Accessed April 19,2024. [Http://Repository.Ng:8080/Jspui/Handle/123456789/897](http://Repository.Ng:8080/Jspui/Handle/123456789/897). Futminna. Edu.
3. Shumieng Zhang, Jiayang Ma, Weiwei wang, Chao Zhang Fengli sun and yajun xi the over expression of the switchgrass (*Panicum Virgatum L.*) genes PvYOC1-N or PvLHY-K affects circadian rhythm and hormone metabolism in transgenic Arabidopsis seedlings (2024).
4. M.M. Manyuchi. Value Addition of Woody Biomass (Saw Dust) to Bioethanol. C.Mbohwa Value Addition of Woody Biomass (Saw Dust) to Bioethanol. E. Muzenda Value Addition of Woody Biomass (Saw Dust) to Bioethanol 2020.
5. Obene, John Justine and Obwola Geoffrey. Bio-Ethanol Production from Cassava Waste Peels using Acid Hydrolysis and Fermentation Process ISSN: 2579-079X.
6. Samuel Kofi Tulashie¹. Ephraim Edem Amoah Akpari¹. Godfred Appiah 2.3. Ebenezer Kelvin Andoh¹ Abigail Adongo¹. Acid hydrolysis of sawdust waste into bioethanol (03 July 2021).
7. Galbe, M., Sassener, P., Wingren, A., Zacchi, G.: process engineering economics of bioethanol production. Biofuels. 303-327(2007)

8. Yussouff Aa. Raji Wa. Ajibade S. Olowo So. Yussouff Aa. The potential Of Cassava Peel and Applicable Technologies for Sustainable Biogas-Bio Ethanol Production. Eng Technol Res J. 2018:3(2):34-41.
Doi:10.47545/Etrj.2018.3.2.044
9. Kumar P. Barrett DM. Delwiche MJ, Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuels production. Ind Eng Chem Res 48:3713-3729. <https://doi.org/10.1021/ie801542g>
10. Leusteam I (2009) Bioethanol from lignocellulosic materials. J Agroalimnt Process Technoln15:94-101
11. Hayes DJ (2009) An examination of biorefining processes, catalysts. And challenges. Catal today 1-45:138-151
12. Nwakaire JN, Ezeoha SL., Ugwushiwu BO (2013) Production of ethanol from cellulosic ethanol from wood sawdust. Agric Eng Int CIGR e-journaln15:136-140
13. Olayemi S, Ibikunle A, Olayemi J (2009) Production of ethanol from cassava and yam peel using acid hydrolysis. Am Sci Res J Eng Technol Sci 52(1):67-78
14. Cao G. Ximenes E. Nichols NN. Zhang L. Ladisch M (2013) Biological abatement of cellulase inhibitors. Bioresour Technol 604-610
15. McMillan, J.D.: Bioethanol production: status and prospects. Renew. Energy. 10. 295-309 (1997)
16. Adeleke, A. E., et al. (2017). "Bioethanol production from cassava peel waste."
17. McLaughlin, S. B., & Kszos, L. A. (2005) "Development of switchgrass as a bioenergy feedstock."
18. Vallejos. M. E... Kruyeniski. J., Area, M. C., Second-generation bioethanol from industrial wood waste of South American species. Biofuel Research Journal. vol. 15. pp. 654-667. 2017. DOI: 10.18331/BRJ2017.4.3.4
19. Balat. M... Balat. H... and Oz. C. Progress in bioethanol processing. Progress in Energy and Combustion Science. vol. 34 (5). pp. 551-573. 2008.