ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bioethanol Production from Agricultural Wastes: A Comparative Study of *Musa acuminata* Peels and *Oryza sativa* Husk.

Shekhar D Tidke[1]*, Subodh A Kamble[1], Anil S Khandagale[1], Shruti S Kulkarni[1] and Sneha H Mendhe[2].

[1]Department of Biotechnology, Dr. D. Y. Patil Arts, Commerce & Science College, Pimpri, Pune, MS, India-411018
[2]Department of Botany, Dr. D. Y. Patil Arts, Commerce & Science College, Pimpri, Pune, MS, India-411018

ABSTRACT

Agricultural waste refers to the residues or parts resulting from the cultivation and processing of raw agricultural products. These wastes possess high lignocellulose biomass potential, which can be utilized for the production of important fuels needed in the future. This study investigates the production of bioethanol from agricultural wastes, specifically banana peels and rice husk, through physical and chemical pretreatment methods. NaOH and H_2SO_4 pretreatments were applied to enhance sugar yield from these lignocellulosic biomasses. Results indicated that acidic pretreatment was more effective, yielding a higher concentration of reducing sugars and a greater bioethanol density compared to alkaline treatment. The maximum recovery of bioethanol was achieved from acidic pretreated banana samples (50%), demonstrating the potential of banana peels as a viable source for bioethanol production. Overall, both agricultural wastes proved to be suitable for bioethanol generation, highlighting their potential as alternative fuel sources. This study reinforces the importance of agricultural waste utilization for sustainable bioethanol production, contributing to the advancement of green energy technologies and promoting a circular economy.

Keywords: Bioethanol, Fermentation, GC-FID, Agricultural wastes, Banana peels and Rice husk.

**Corresponding author*

ISSN: 0975-8585

INTRODUCTION

Bioethanol derived from sustainable sources is being explored as a potential alternative to fossil fuels. Valuable products such as fuel, ethanol and biodiesel can be produced from lignocellulosic biomass, including wood and agricultural residues [1]. These renewable materials offer the potential to replace harmful fossil fuels and promote environment friendly products. Fermentation of ethanol, being CO_2 neutral, does not contribute negatively to the greenhouse effect [2, 3]. C_2H_6O is the chemical formula for ethanol, which is a clear, combustible fluid. It is widely used as a solvent, fuel, and in the production of other compounds. It is also used as an antiseptic, for heating, and in alcoholic drinks. Bioethanol, which is mostly made from corn and sugarcane in the US and Brazil, is the most popular renewable transportation fuel. It has benefits such as less dependence on crude oil, cleaner burning, and less toxicity [4]. The depletion of fossil fuels has increased interest in bioethanol as an alternative energy source. Although sugarcane molasses is used to make ethanol in India, there are major issues with its scarcity and price. Despite the fact that cellulosic resources are plentiful and less expensive, their conversion to ethanol is complicated and expensive. As a result, innovative strategies are necessary for utilizing renewable resources like fruit waste. After rice, maize, and milk, bananas are the fourth most important food item in the world [5-8]. Although it is difficult to create ethanol from lignocellulosic resources like corncob, cornstalk, cornhusk, sugarcane bagasse, and sugarcane bark, it may take the place of bio-ethanol produced from food crops. This approach makes use of a renewable energy source that is readily accessible. It prevents competition with food supplies and helps us utilize agricultural land more effectively [9]. Pre-treatment, hydrolysis of straw to produce simple sugars, anaerobic fermentation to produce ethanol, and distillation are all steps in the manufacturing of bioethanol. Pre-treatment aims to enhance cellulose structure for improved enzymatic access [10]. In alkaline pretreatment, lignocellulosic materials are mixed with bases like sodium, potassium, calcium, and ammonia at specific temperatures and pressures. This method aids in the breakdown of portions of the material, which causes lignin to be disrupted, cellulose to expand, and decrystallization to occur. The pH is also lowered by the organic acids generated during alkaline treatment, which also pulls out hemicelluloses. The outcome of this is a wet solid fraction that is mostly cellulose and a liquid fraction that includes dissolved hemicelluloses, lignin, and trace amounts of remaining inorganic compounds. They are rinsed with warm or hot water to get rid of any residual compounds and enzyme inhibitors after the solids have been separated. The treated solids' sugar release is enhanced by this washing step [11]. Dried biomass is ground, occasionally submerged in water, and then subjected to an acidic environment at a specific temperature for a specific period during acidic pretreatment. The liquid and solid components of the mixture are separated by filtration, and the solid components are then washed or neutralized before the sugar is extracted. Sulfuric and phosphoric acids are frequently used for hydrolysis due to their cost-effectiveness and efficiency in breaking down lignocellulose [12]. However, more acidic solutions enhance hydrolysis. The fermentation process is a widely used, traditional, and well-established natural metabolic pathway for converting lignocellulosic biomass into bioethanol, in which an organism breaks down complex carbohydrates into simple sugars and then converts the sugars into either an alcohol or an acid. Yeast, bacteria, or enzymes are used in this fermentation process as an experiment [13, 14]. The disintegration of starch molecules into smaller molecular sugars that can be fermented by the traditional method is known as hydrolysis. Acid hydrolysis and enzymatic hydrolysis are the two types of hydrolysis processes. The chemical bonds are broken during the acid hydrolysis using a powerful acid, such sulfuric acid (H_2SO_4) or hydrochloric acid (HCl). Enzymatic hydrolysis, the second process, uses enzymes to break the starch molecules' chemical bonds. To make the enzymes highly selective, i.e., acting almost exclusively on the links between the monomers that make up the starch [15, 16], they are chosen in accordance with the vegetable source to be employed. Distillation is a physical process, as opposed to a chemical one, that separates mixtures according to variations in the volatility of its constituent components. Because ethanol has a lower vapor pressure than water, distilleries may concentrate ethanol produced by fermentation, by heating the mixture [17]. Ethanol is an organic solvent with unique physicochemical and pharmacological properties appropriate for pharmaceutical manufacturing. It is also employed as a disinfectant and in the pharmaceutical production of marjoram ointment [18, 19]. Due to the growing demand for ethanol, there has been a greater global interest in finding alternate sources for its production [5, 20].

MATERIALS AND METHODS

Collection of microbial cultures and agricultural waste

Banana peels were sourced from the fruit market in Paud region of Pune, while rice husk was obtained from the rice mill in Karmoli, Paud, Pune. Cultures of *Saccharomyces cerevisiae* (baker's yeast) and *Trichoderma reesei* (NFCCI-2242) were procured from the Department of Microbiology, Dr. D. Y. Patil Arts, Commerce, and Science College, Pimpri, Pune-18.

Pretreatment of banana peels and rice husk

Physical Pretreatment: - The collected rice husk samples were cleaned and ground using a grinding machine to obtain homogenized, small-sized particles suitable for further study [21-23].

Alkaline Pretreatment: - Banana peels (40 g) were mixed with 240 ml of 10% NaOH in a 6:1 ratio, while rice husk samples were subjected to alkaline treatment using three different concentrations of NaOH (1%, 2%, and 3%). The banana peels were heated at 120°C for six hours, whereas the rice husk samples underwent autoclaving at 121°C and 15 psi. Following the treatment, the mixtures were filtered to separate the solid residues from the filtrate. The solid residues were then thoroughly washed with tap water and subsequently rinsed with distilled water to achieve a neutral pH. The banana peel residues were air-dried at 45°C, while the rice husk residues were stored at 4°C in a refrigerator for future use. The filtrate solution was preserved for DNSA analysis to assess the concentration of reducing sugars present in the sample [21, 24, 25]. To ensure accuracy and reproducibility, all experiments were conducted in triplicate.

Acidic Pretreatment: - Banana peels (40 g) were treated with 200 ml of 5% H_2SO_4, while rice husk samples underwent acidic pretreatment at three different H_2SO_4 concentrations (1%, 2%, and 3%). The banana peels were heated at 120°C for six hours, whereas the rice husk samples were autoclaved at 121°C and 15 psi. Following the treatment, both mixtures were filtered using muslin cloth to separate the solid residues from the filtrate. The solid residues were then gently washed with tap water, followed by distilled water, to achieve a neutral pH. The banana peel residues were air-dried at 45°C, while the rice husk residues were stored at 4°C in a refrigerator for future use. The filtrate solution was preserved for DNSA analysis to quantify the concentration of reducing sugars [21, 24, 26]. To ensure accuracy and reproducibility, all experiments were conducted in triplicate.

Hydrolysis of Banana Peel Samples: - A 10% sulfuric acid solution was prepared and mixed with the lignocellulosic biomass derived from banana waste following various pretreatment processes. The mixture was maintained at a sulfuric acid-to-fiber ratio of 6:1. The setup was then heated at 120°C for six hours and subsequently allowed to cool [24].

Glucose Assay and Dinitrosalicylic Acid (DNSA) Test of banana peels and rice husk

The hydrolyzed banana peel samples were subjected to a glucose assay to estimate the presence of reducing sugars. A few drops of Benedict's solution were added, and the resulting color changes were observed at intervals of 0, 24, 48, and 72 hours. The Benedict's reaction provides a semi-quantitative assessment, with distinct color transitions indicating sugar concentration: blue signifies no sugar, green corresponds to 0.5% sugar, yellow represents 1% sugar, orange denotes 1.5% sugar, red indicates 2% sugar, and brown signifies the highest sugar concentration [24]. Meanwhile, the rice husk samples underwent a dinitrosalicylic acid (DNSA) test to quantify reducing sugars. A 1 ml aliquot of the sample was placed in a clean test tube, followed by the addition of 2 ml of DNS reagent. The mixture was heated in a boiling water bath for five minutes and subsequently allowed to cool. Once cooled, 7 ml of distilled water was added to dilute the solution, and absorbance was measured using a UV spectrometer at 540 nm, with a blank sample serving as the control [27]. This method ensures precise quantification of reducing sugars in both banana peel and rice husk samples.

Fermentation of banana peels and rice husk

The fermentation process involved two distinct experimental setups. For banana peel fermentation, *Saccharomyces cerevisiae* cells were suspended in deionized water, with pretreated banana

waste serving as the sole carbon source. Among four bottles used, two acted as controls containing only deionized water and banana peels, while the remaining three contained both yeast cells and banana peels in deionized water. Fermentation was carried out for three days, as *S. cerevisiae* has a typical growth period of three days [24, 28]. Simultaneously, saccharification and fermentation of rice husk were conducted in 1000 ml of basal media, with its pH adjusted to 5.5–6.0 and autoclaved at 121°C and 15 psi for 15 minutes. Post-autoclaving, 5% dextrose was added, and 100 ml of the sterilized media was distributed into 250 ml flasks containing pretreated rice husk samples. *Trichoderma reesei* (100 µl) was inoculated under sterile conditions, and flasks were incubated at 28±2°C on a rotary shaker at 120 rpm for 48 hours. DNSA analysis was performed to estimate sugar content. After 72 hours, *S. cerevisiae* was introduced into the same flasks to initiate fermentation, which continued under identical conditions for another 72 hours, with sampling conducted every 48 hours for reducing sugar estimation [21, 29, 30].

Filtration and distillation process of banana peels and rice husk

Samples were then filtered by using muslin cloth to separate the solid substrate from liquid and then distillation was done at 78.37°C to get the ethanol samples for GC analysis. Percentage recovery of bioethanol obtained in the from distillate was calculated using the following formula [17, 31],

$$\text{Percent recovery} = \frac{\text{Amount of substance recoverd on purification}}{\text{Amount of substance originally taken}} \times 100$$

Analytical test for bioethanol produce from banana peels and rice husk

Density of bioethanol produce: -Bioethanol was transferred to measuring cylinder set to zero reading on electronic balance. The weight and volume of ethanol was recorded and the density was calculated using the formula [32],

$$\text{Density } \left(\frac{\text{g}}{\text{ml}}\right) = \frac{\text{mass ethanol}}{\text{Volume of ethanol}}$$

pH Test of bioethanol produce: - The pH meter was first inserted in a buffer solution to standardize the apparatus then placed into the sample (ethanol) and the reading was recorded [32].

Identification of bioethanol produce: - 5 ml distillate sample was taken. A pinch of potassium dichromate and a few drops of H_2SO_4 as indicated in colour. The colour changes were recorded from transparent to green [33].

Lignin estimation test: - The weight of untreated banana peels was taken and then weight of samples was measured after alkaline and acidic pretreatment. The samples were washed with the distilled water and then dried completely to measure the weight of the samples [21].

$$\text{Lignin \%} = \frac{\text{Lignin weight}}{\text{Substrate weight}} \times 1oo$$

GC-FID analysis of bioethanol from banana peels and rice husk

Analysis of ethanol was conducted using SRI GC model 8610C, equipped with a 60 m column (Restec MXT-1, Id 0.53 mm, 5 µM), on column injector and FID conditions:250°C; H_2, 25 psi, equivalent to 100 ml/min., gain set to medium. The GC was also equipped with an internal air compressor and hydrogen generator. N_2 was used as carrier gas with pressure control (24 psi constant, equivalent to 27 ml/min). The GC was connected to a computer running peak simple software version 2.8. Oven temperature (and hence column and injector temperature) was initially set at 50°C and then elevated at the rate of 7°C/min to 100°C, thus giving a total run time of 7 min. Furthermore, 2 µl sample was injected manually at time 0, using a 5 µl Hamilton syringe and temperature cycle was started. Syringe was thoroughly washed with ethyl acetate between injection to avoid cross-contamination. Each injection was repeated three times, ethanol routinely came out at retention time equivalent to 65°C [34, 35].

ISSN: 0975-8585

RESULTS AND DISCUSSION

Pretreatment of banana peels and rice husk

The pretreatment of banana peels and rice husk involved chemical processing to enhance biomass breakdown. Banana peels were treated with either NaOH or H_2SO_4 and subjected to cooking at 120°C for six hours. The NaOH treatment facilitated lignin dissolution, while H_2SO_4 treatment led to the solubilization of both hemicellulose and lignin. This process enabled the subsequent hydrolysis, converting polysaccharides into monosaccharides. Similarly, rice husk was treated with varying concentrations of NaOH (1%, 2%, 3%) and cooked under identical conditions to dissolve lignin. Additionally, rice husk samples were treated with different concentrations of H_2SO_4 (1%, 1.5%, 2%), promoting hemicellulose and lignin solubilization. Hydrolysis further degraded polysaccharides into monosaccharides, facilitating efficient biomass conversion.

Glucose assay and Dinitrosalicylic acid (DNSA) test of banana peels and rice husk

Glucose assay of banana peels

The glucose assay of hydrolyzed banana samples was conducted to determine the presence of reducing sugars in pretreated banana peels. The assay relied on Benedict's reaction, where color changes provided a semi-quantitative estimation of sugar content over a 72-hour period. The results indicated that in alkaline conditions, the color remained consistently blue, confirming the absence of reducing sugars throughout the experiment. Conversely, in acidic conditions, an initial green coloration at 0 and 24 hours corresponded to a reducing sugar concentration of 0.5%. By 48 hours, the acidic sample transitioned to brown, signifying an increase in reducing sugar content to over 2%, which was maintained at 72 hours. These observations highlight that acidic hydrolysis effectively facilitated sugar release from banana peels, whereas alkaline treatment did not yield detectable amounts of reducing sugar. Determination of reducing sugar of hydrolysed banana sample is depicted in table 1.

Table 1. Determination of reducing sugar of hydrolyzed banana sample

Sr. No.	Time (in hours)	Colour		Amount (in %)	
		Alkaline	Acidic	Alkaline	Acidic
1	0	Blue	Green	No Sugar	0.5
2	24	Blue	Green	No Sugar	0.5
3	48	Blue	Brown	No Sugar	More than 2
4	72	Blue	Brown	No Sugar	More than 2

DNSA Analysis of Pretreated Rice Husk Samples

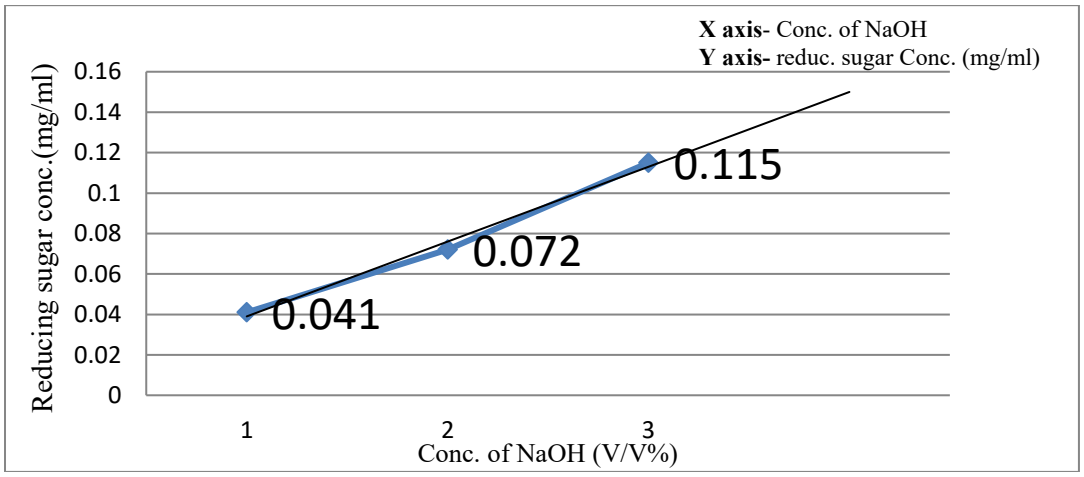
The reducing sugar content in rice husk samples subjected to alkaline and acidic pretreatment was estimated using DNSA analysis, as represented in Graph No. 1 & 2.

Alkaline Pretreatment of Rice Husk Samples: - The reducing sugar content in rice husk samples treated with alkaline solutions varied with sample concentration, as detailed in Table 2. The highest reducing sugar concentration (0.115 mg/ml) was observed in the sample treated with 3% NaOH, indicating that increasing alkaline concentration facilitated higher sugar release.

ISSN: 0975-8585

Table 2: Reducing sugar content in alkaline pretreated rice husk sample

Sr. No.	Sample Concentration (%)	Reducing sugar conc. (mg/ml)
1	1	0.041
2	2	0.072
3	3	0.115

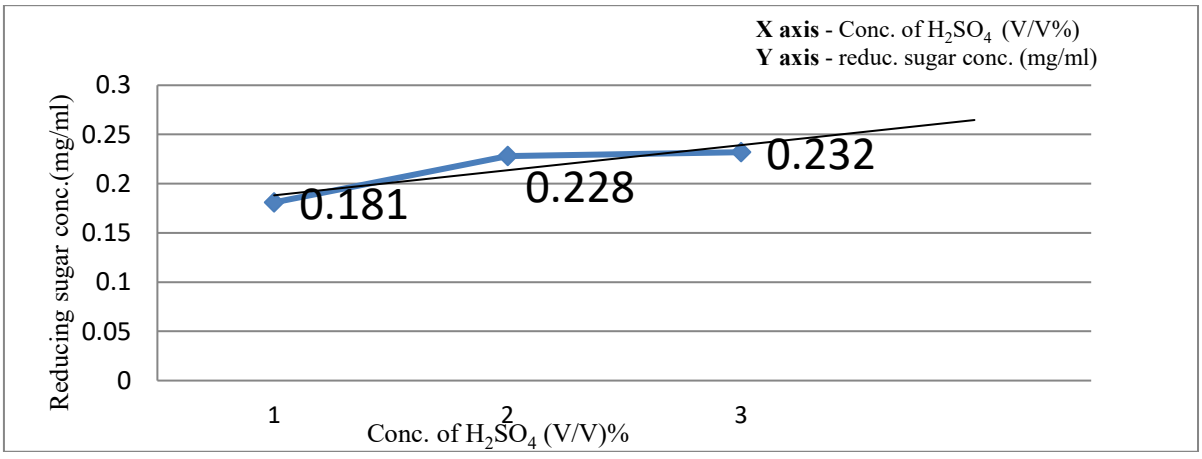


Graph 1: Estimation of reducing sugars after alkaline pretreatment

Acidic Pretreatment of Rice Husk Samples: - Similarly, rice husk samples subjected to acidic pretreatment showed varying levels of reducing sugar content, as presented in Table 3. The **2% H_2SO_4 pretreated sample** exhibited the maximum reducing sugar concentration (0.232 mg/ml), suggesting that acidic hydrolysis at this concentration was most effective in enhancing sugar yield.

Table 3: Reducing sugar content in acidic pretreated sample rice husk

Sr. No.	Sample Concentration (%)	Reducing sugar conc. (mg/ml)
1	1	0.181
2	1.5	0.228
3	2	0.232



Graph 2: Estimation reducing sugars after acidic pretreatment

ISSN: 0975-8585

Bioethanol Production from Banana Peels and Rice Husk

The bioethanol yield was successfully obtained following the filtration and distillation process, as illustrated in Fig. 1 and Fig. 2. The recovery percentage of bioethanol from banana peel samples varied based on the pretreatment method. The acidic treatment resulted in a yield of 50%, whereas the alkaline treatment produced 45% recovery. Similarly, the bioethanol yield from rice husk samples was influenced by the pretreatment method. The acidic treatment yielded 46%, while the alkaline treatment resulted in a recovery percentage of 40%.

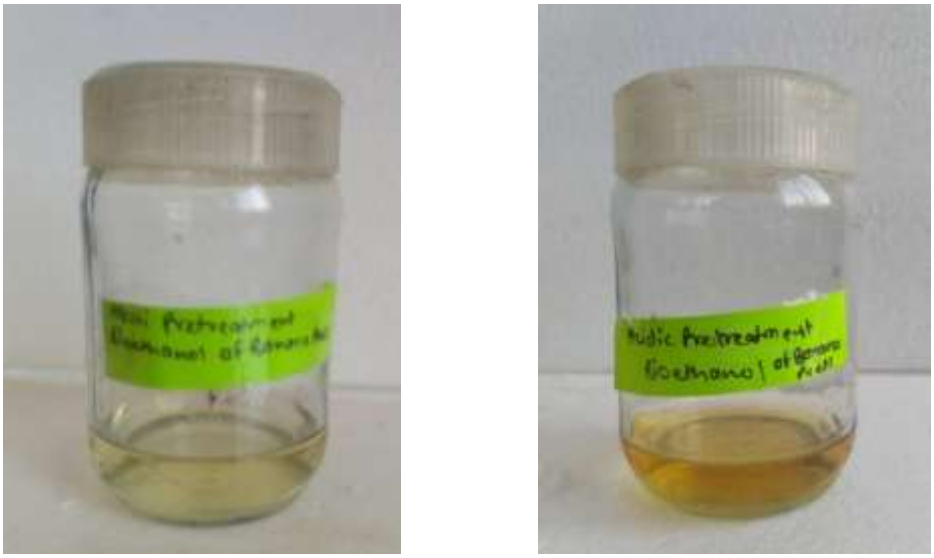


Figure 1: Bioethanol produces from alkaline and acidic pretreated banana peels where, A is alkaline pretreated and B is acidic pretreated bioethanol

Figure 2: Bioethanol produces from alkaline and acidic pretreated rice husk where, A is alkaline pretreated and B is acidic pretreated bioethanol.

Analytical testing of Bioethanol produced from Banana peels and rice husk

The analytical assessment of bioethanol derived from banana peels and rice husk included the evaluation of density, pH, and identification using potassium dichromate.

Analytical testing of Bioethanol produced from Banana peels

Density of Bioethanol produced from Banana peels: - The density of bioethanol varied based on the pretreatment method applied to banana peels (Table 4). The density of bioethanol extracted through alkaline pretreatment was 1.12 g/ml, whereas the bioethanol obtained from acidic pretreatment had a density of 2.12 g/ml. This indicates that acidic pretreatment resulted in a bioethanol sample with higher density compared to the alkaline method.

Table 4: Density of bioethanol produced from banana peels

Sr. No.	Density of bioethanol from banana peels (g/ml)	
	Alkaline pretreatment	Acidic pretreatment
1	1.12	2.12

pH Test of Bioethanol produced from Banana peels: - The pH of bioethanol samples also differed according to the pretreatment method (Table 5). Bioethanol obtained through alkaline pretreatment exhibited a pH of 1.65, while that produced via acidic pretreatment had a pH of 4.67. Both samples were found to be acidic in nature, with the acidic pretreated bioethanol showing a significantly higher pH value.

Table 5: pH of bioethanol produced from banana peels

Sr. No.	pH test of bioethanol from banana peels	
	Alkaline pretreatment	Acidic pretreatment
1	1.65	4.67

Identification of Bioethanol using potassium dichromate test: - The presence of bioethanol in the distilled sample was confirmed using potassium dichromate reagent, which induced color changes during the reaction. Initially, the sample transitioned from transparent to pink and subsequently from pink to green, indicating the presence of bioethanol in the distillate.

Lignin estimation in pretreated Banana peel: - The lignin content in alkaline and acidic pretreated banana peel samples was measured to assess the effectiveness of delignification (Table 6). The results indicated that acidic pretreatment was significantly more effective in lignin removal compared to alkaline pretreatment. The alkaline pretreated sample contained 56.4% lignin, whereas the acidic pretreated sample exhibited a lower lignin content of 37.2%, demonstrating enhanced delignification through acidic treatment.

Table 6: Lignin content in banana peels

Sr. No.	Lignin content in banana peels (%)	
	Alkaline pretreatment	Acidic pretreatment
1	56.4	37.2

Analytical testing of Bioethanol produced from Rice Husk

Density of Bioethanol Produced from Rice Husk: - The density of bioethanol varied based on the pretreatment method applied to rice husk (Table 7). The bioethanol extracted through alkaline pretreatment had a density of 0.964 g/ml, whereas the bioethanol obtained from acidic pretreatment exhibited a density of 0.999 g/ml. This suggests that acidic pretreatment resulted in a bioethanol sample with higher density compared to the alkaline method.

Table 7: Density of bioethanol produced from rice husk

Sr. No.	Density of bioethanol produced from rice husk (g/ml)	
	Alkaline	Acidic
1	0.964	0.999

pH Test of Bioethanol produced from Rice Husk: - The pH of bioethanol samples also differed according to the pretreatment method (Table 8). Bioethanol obtained through alkaline pretreatment exhibited a pH of 2.25, while that produced via acidic pretreatment had a pH of 4.57. Both samples were found to be acidic in nature, with the acidic pretreated bioethanol showing a significantly higher pH value.

Table 8: pH of bioethanol produced from rice husk

Sr. No.	pH test of bioethanol produced from rice husk	
	Alkaline	Acidic
1	2.25	4.57

Identification of Bioethanol using potassium dichromate test: - The presence of bioethanol in the distilled sample was confirmed using potassium dichromate reagent, which induced color changes during the reaction. Initially, the sample transitioned from transparent to pink and subsequently from pink to green, indicating the presence of bioethanol in the distillate.

Lignin Estimation in Pretreated Rice Husk: - The lignin content in alkaline and acidic pretreated rice husk samples was measured to assess the effectiveness of delignification (Table 9). The results indicated that acidic pretreatment was more effective in lignin removal compared to alkaline pretreatment. The alkaline pretreated sample contained 91.3% lignin, whereas the acidic pretreated sample exhibited a lower lignin content of 87.5%, demonstrating enhanced delignification through acidic treatment.

Table 9: Lignin content in rice husk

Sr. No.	Lignin content in rice husk (%)	
	Alkaline	Acidic
1	91.3	87.5

Comparative Analysis of Bioethanol Production from Banana Peels and Rice Husk

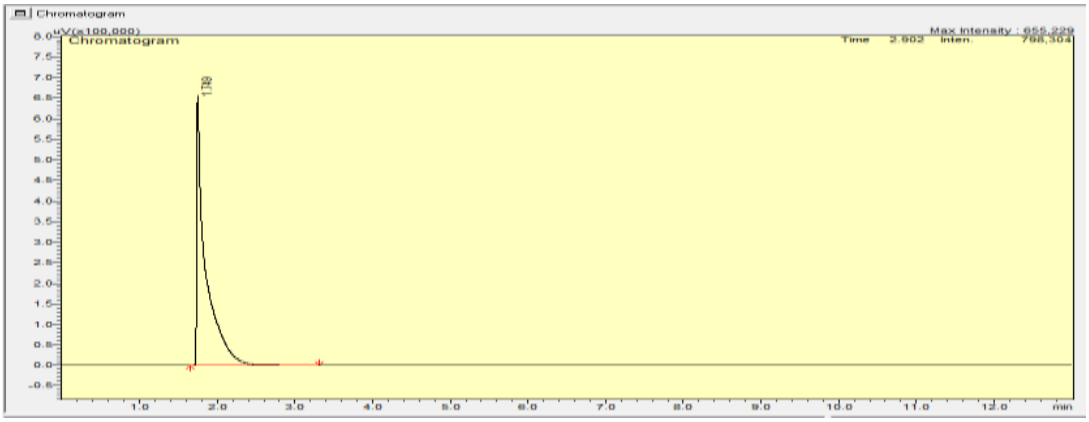
The comparative analysis of bioethanol production from banana peels and rice husk after alkaline and acidic pretreatment reveals significant differences in yield, density, pH, and lignin content, emphasizing the effectiveness of each biomass type. Bioethanol recovery was notably higher in banana peels, reaching 50% through acidic pretreatment, compared to 46% for rice husk under the same conditions, while alkaline pretreatment resulted in lower yields for both. Density measurements indicated that bioethanol from banana peels had a significantly higher value (2.12 g/ml) than that from rice husk (0.999 g/ml), with acidic pretreatment yielding the densest bioethanol in both cases. Similarly, the pH of bioethanol was greater in banana peels (4.67) compared to rice husk (4.57) under acidic treatment, whereas alkaline pretreatment led to notably lower pH levels. Additionally, acidic pretreatment was more effective in lignin removal, with banana peels containing only 37.2% lignin after treatment, significantly lower than the 87.5% found in rice husk, suggesting that banana peels may be a more suitable biomass for efficient bioethanol production.

ISSN: 0975-8585

GC-FID analysis of Bioethanol produced from alkaline and acidic pretreated Banana peel and Rice husk

GC-FID Analysis of Alkaline Pretreated Banana Peels

The gas chromatography with flame ionization detection (**GC-FID**) chromatogram obtained from the alkaline pretreated banana sample validated the presence of bioethanol, with a primary peak detected at a retention time of 1.749 minutes (Graph 3 and Table 10). Two distinct components were identified in the sample, with their retention time, area, and peak height recorded as follows:



Graph 3: Ethanol estimation from alkaline pretreated banana peels sample

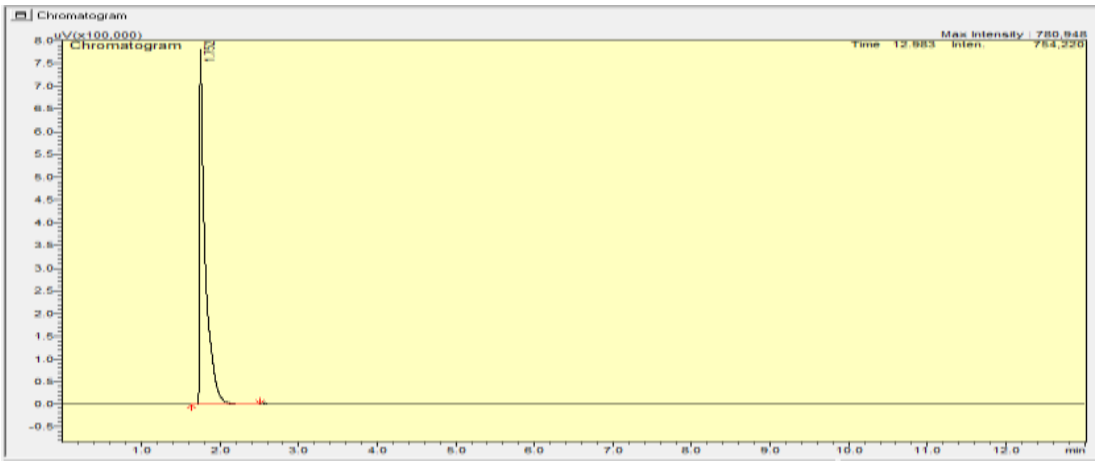
The dominant peak at 1.749 min indicates the presence of bioethanol, suggesting that the alkaline pretreatment process successfully facilitated ethanol extraction.

Table 10: GC-FID graph representing peak values of alkaline pretreated banana peels sample

Peak No.	Retention Time (min)	Area	Height
1	1.749	515689	653425
2	3.355	3552	961
Total	—	5160371	654386

GC-FID Analysis of Acidic Pretreated Banana Peels

Similarly, the chromatogram obtained from the acidic pretreated banana sample confirmed bioethanol presence, with a primary peak observed at a retention time of 1.752 minutes (Table 11 and Fig. 4). Two components were detected, as detailed below:



Graph 4: Ethanol estimation from acidic pretreated banana peels sample

The peak at 1.752 min confirms bioethanol presence in the acidic pretreated sample, indicating a successful ethanol recovery process.

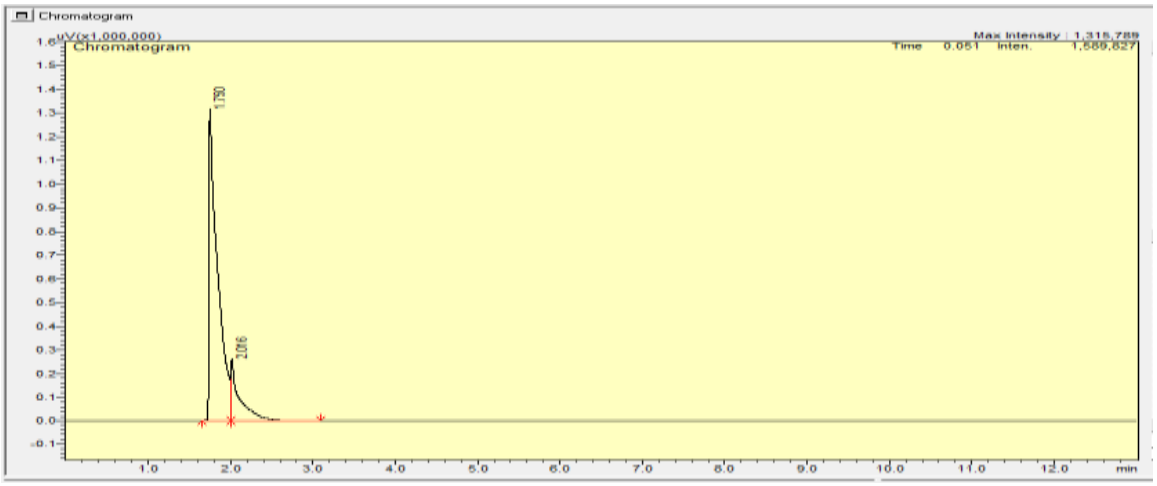
Table 11: GC-FID graph representing peak values of acidic pretreated banana peels sample

Peak No.	Retention Time (min)	Area	Height
1	1.752	3927539	774359
2	2.567	6735	3125
Total	—	3934274	777484

In comparative analysis found that, the retention times for bioethanol in both samples were comparable, at 1.749 min for alkaline pretreatment and 1.752 min for acidic pretreatment. The area and height values were significantly higher in the acidic pretreated sample, suggesting that acidic hydrolysis resulted in enhanced ethanol extraction compared to alkaline treatment.

GC-FID Analysis of Alkaline Pretreated Rice Husk

The chromatogram obtained from the alkaline pretreated rice husk sample confirmed the presence of bioethanol, with a primary peak detected at a retention time of 1.750 minutes (Graph 5 and Table 12). Three distinct components were identified in the sample, with their respective retention time, area, and peak height recorded as follows:



Graph 5: Bioethanol estimation from alkaline pretreated rice husk sample

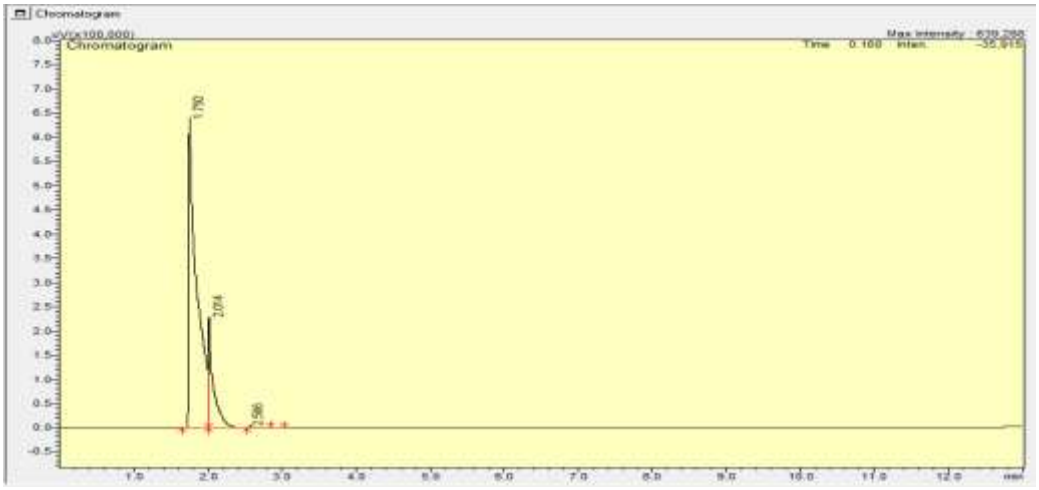
The dominant peak at 1.750 min confirms bioethanol presence, indicating that alkaline pretreatment effectively facilitated ethanol extraction from rice husk.

Table 12: GC graph representing peak values of alkaline pretreated rice husk sample

Peak No.	Retention Time (min)	Area	Height
1	1.750	9,522,778	1,309,468
2	2.016	1,965,822	259,651
3	7.201	9,893	1,341
Total	—	11,498,493	1,570,460

GC-FID Analysis of Acidic Pretreated Rice Husk

Similarly, the chromatogram obtained from the acidic pretreated rice husk sample validated the presence of bioethanol, with the primary peak detected at a retention time of 1.750 minutes (Graph 6 and Table 13). Three components were identified, with their retention time, area, and peak height presented below:



Graph 5: Bioethanol estimation from acidic pretreated rice husk sample

The peak at 1.750 min confirms bioethanol presence in the acidic pretreated rice husk sample, demonstrating successful ethanol recovery through acidic hydrolysis.

Table 13: GC graph representing peak values of alkaline pretreated rice husk sample

Peak No.	Retention Time (min)	Area	Height
1	1.750	4,726,032	636,847
2	2.014	1,065,174	225,646
3	2.566	11,505	3,378
Total	—	5,802,711	865,871

In comparative analysis found that, the retention times for bioethanol were consistent across both pretreatment methods, with a primary peak detected at 1.750 minutes. The area and height values were significantly higher in the alkaline pretreated sample, suggesting greater ethanol concentration compared to acidic pretreatment. Acidic pretreatment yielded a lower overall intensity, indicating potential differences in bioethanol purity or extraction efficiency.

CONCLUSION

This study successfully demonstrated the bioethanol production potential of banana peels (*Musa acuminata*) and rice husk (*Oryza sativa*) through alkaline and acidic pretreatment methods. The findings revealed that acidic pretreatment was significantly more effective, resulting in higher reducing sugar concentrations, greater bioethanol yield, and enhanced lignin removal compared to alkaline treatment. Bioethanol recovery was highest in acidic pretreated banana peels (50%), followed by acidic pretreated rice husk (46%), highlighting banana peels as a more efficient biomass source. Additionally, GC-FID analysis confirmed the presence of ethanol, with peak retention times consistently observed in both biomass types, further validating their suitability for bioethanol production.

ACKNOWLEDGEMENT

The authors extend their gratitude to the Principal of Dr. D. Y. Patil Arts Commerce and Science College, Pimpri, Pune for their continuous support in carrying out this work. We also express our heartfelt thanks to our parents for their boundless encouragement and motivation.

REFERENCES

[1] Ingale, S., Joshi, S., & Gupte, A. (2014). Production of bioethanol using agricultural waste: Banana pseudo stem. Journal of Microbiology, 45(3), 885-892. https://doi.org/10.1590/s1517-83822014000300018

[2] Yoshwathana, N., Phuriphipat, P., Treyawutthiwat, P., & Eshtiaghi, M. (2010). Bioethanol production from rice straw. Journal of Energy Research, 1(1), 26-31. https://doi.org/10.3844/erjsp.2010.26.31
[3] Balat, M. (2007). Global biofuel processing and production trends. Journal of Energy Exploration and Exploitation, 25(3), 195-218. https://doi.org/10.1260/014459807782009204
[4] Hongbo, D., Raghava, R., & Ziaul, H. (2016). Assessment of bioethanol applications on transportation vehicles in Houston. Journal of Fundamental of Renewable Energy and Applications, 6, 207. https://doi.org/10.4172/2090-4541.1000207
[5] Sharma, P., & Mishra, A. (2015). Biofuel production from banana peel by using microwave. Journal of Science, Engineering and Technology, 3(4), 2395-4752.
[6] Madian, H., Abo-State, M., Ragab, A., EL-Gendy, N., & Farahat, L. (2014). Bioethanol production from rice straw enzymatically saccharified by fungal isolates, Trichoderma viride F94 and Aspergillus terreus F98. Journal of Scientific Research, 3, 19-29. https://doi.org/10.4236/soft.2014.32003
[7] Nutawan, Y., Phattayawadee, P., Pattranit, T., & Mohammad, N. (2010). Bioethanol production from rice straw. Journal of Energy Research, 1(1), 26-31. https://doi.org/10.3844/erjsp.2010.26.31
[8] Souza, E., Liebl, G., Marangoni, C., Sellin, N., Montagnolia, M., & Souza, O. (2014). Bioethanol from fresh and dried banana plant pseudostem. Journal of Association Sciences, 5(3), 769-776. https://doi.org/10.3303/CET1438046
[9] Braide, W., Kanu, I., Oranusi, U., & Adeleye, S. (2016). Production of bioethanol from agricultural waste. Journal of Fundamental and Applied Sciences, 8(2), 372-386. https://doi.org/10.4314/jfas.v8i2.14
[10] Sokhansanj, S., Mani, S., & Turhollow, A. (2006). Economics of producing fuel pellets from biomass. Journal of Applied Engineering in Agriculture, 22(3), 421-426. https://doi.org/10.13031/2013.20447
[11] Bensah, D., & Mensah, M. (2013). Chemical pretreatment methods for the production of cellulosic ethanol: Technologies and innovations. International Journal of Chemical Engineering, 21. https://doi.org/10.1155/2013/719607
[12] Demirbas, A. (2009). Biofuels from agricultural biomass. Journal of Energy Sources, 31, 1573-1582. https://doi.org/10.1080/15567030802094011
[13] Hossain, N., & Jalil, R. (2015). Analyses of bio-energy properties from Malaysian local plants: Sentang & Sesenduk. Asia Pacific Journal of Energy and Environment, 2(3), 141-144.
[14] Cutzu, R., & Bardi, L. (2017). Production of bioethanol from agricultural wastes using residual thermal energy of a cogeneration plant in the distillation phase. Journal of Fermentation, 3(24), 1-8. https://doi.org/10.3390/fermentation3020024.
[15] Gomes, C., Quintero, A., & Paz, C. (2018). Production of bioethanol from sugarcane bagasse: Status and prospective. Journal of Bioresource Technology, 101(13), 4754-66. https://doi.org/10.1016/j.biortech.2009.10.097
[16] Borglum, G. (2010). Starch hydrolysis for ethanol production. Journal of Miles Laboratories Inc., Industrial Products Group, 264-269. https://doi.org/10.1260/014459807782009204
[17] Hall, L., & Reuter, P. (2007). Compositional analysis of lignocellulosic materials: Evaluation of methods used for sugar analysis of waste paper and straw. Journal of Bioresource Technology, 98(16), 3026-3036. https://doi.org/10.1016/j.biortech.2006.10.013
[18] Gdula-Argasi-Ska, J., Hubicka, U., & Jaskiewicz, J. (2009). Development and validation of GC-FID methods for the determination of ethanol residue in majoramointment. Journal of Polish Pharmaceutical Society, 66(6), 611-615.
[19] Tiscione, N., Alford, I., Yeatman, D., & Shan, X. (2011). Ethanol analysis by headspace gas chromatography with simultaneous flame-ionization and mass spectrometry detection. Journal of Analytical Toxicology, 35, 501-511. https://doi.org/10.1093/anatox/35.7.501
[20] Sarkar, N., Ghosh, S., Bannerjee, S., & Aikat, K. (2012). Bioethanol production from agricultural wastes: An overview. Journal of Renewable Energy, 37, 19-27. https://doi.org/10.1016/j.renene.2011.06.045
[21] Kaur, R., & Singh, H. (2017). Bio-ethanol production from rice husk using simultaneous saccharification and fermentation and optimization of pretreatment methods. Journal of Der Pharma Chemica, 9(7), 1-7.
[22] Belal, E. (2013). Bioethanol production from rice straw residues. Journal of Microbiology, 44(1), 225-234. https://doi.org/10.1590/S1517-83822013000100033

[23] Gonwi, S., Choi, S., Kim, K., Kim, H., & Jong Bae, H. (2013). Bioethanol production from rice straw by popping pretreatment. Journal of Biotechnology for Biofuels, 6(1), 166. https://doi.org/10.1186/1754-6834-6-166

[24] Gaddafi, I., Auwal, M., Abdullahi, A., & Bashir, J. (2016). Bioethanol production from banana peels. Journal of Environmental Science, 10(6), 56-62.

[25] Chantawongsa, N., & Kongkiattikajorn, J. (2013). Pretreatment methods for banana peel as a substrate for the bioproduction of ethanol in SHF and SSF. International Journal of the Computer, the Internet and Management, 21(2), 15-19.

[26] Hossain, A., Ahmed, S., Ahmed, M., Faris, M., Annuar, M., Hadeel, M., & Norah, H. (2011). Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy. African Journal of Microbiology Research, 5(6), 586-598. https://doi.org/10.5897/AJMR10.231

[27] Nwogwugwu, N., Abu, G., & Akaranta, O. (2016). Reducing sugars from agro-waste biomass suitable for industrial applications. Journal of Archives of Applied Science Research, 8(8), 37-40.

[28] Nachaiwieng, W., Lumyong, S., Pratanaphon, R., Yoshioka, K., & Khanongnuch, C. (2015). Potential in bioethanol production from various ethanol fermenting microorganisms using rice husk as substrate. Journal of Biodiversitas, 16(2), 320-326. https://doi.org/10.13057/biodiv/d160229

[29] Afifi, M., Massoud, O., & El-Akasher, Y. (2015). Bioethanol production by simultaneous saccharification and fermentation using pretreated rice straw. American Journal of Microbiological Research, 5(4), 86-90. https://doi.org/10.5402/2013/916481

[30] Pranavya, A., Saravanamurugan, C., & Rajendran, S. (2015). An enzymatic study of bioethanol production using agricultural wastes by Saccharomyces cerevisiae and Zymomonas mobilis. International Journal of Pharma Sciences and Research, 6(2).

[31] Behera, S., Ray, R., & Mohanty, R. (2010). Comparative study of bioethanol production from mahula (Madhuca latifolia L.) flowers by immobilized cells of Saccharomyces cerevisiae and Zymomonas mobilis in calcium alginate beads. Journal of Scientific and Industrial Research, 69, 472-475. https://doi.org/10.1016/j.apenergy.2009.05.030

[32] Ademiluyi, F., & Mepba, H. (2012). Yield and properties of ethanol biofuel produced from different whole cassava flours. International Scholarly Research Notices Biotechnology, 2013, 6. https://doi.org/10.5402/2013/916481

[33] Caputi, A., Veda, M., & Brown, T. (1959). Spectrophotometric determination of ethanol in wine. Journal of Enology Viticulture, 19, 160-165. https://doi.org/10.5344/ajev.1968.19.3.160.

[34] Gerchman, Y., Schnitzer, A., Gal, R., Mirsky, N., & Chinkov, N. (2011). A simple rapid gas-chromatography flame-ionization-detector (GC-FID) method for the determination of ethanol from fermentation processes. Journal of Biotechnology, 11(15), 3612-3616. https://doi.org/10.5897/AJB11.3143

[35] Lachenmeier, D., Godelmann, R., Steiner, M., Ansay, B., & Weigel, J. (2010). Rapid and mobile determination of alcoholic strength in wine, beer, and spirits using a flow-through infrared sensor. Journal of Chemistry Central, 4, 1-10. https://doi.org/10.1186/1752-153X-4-5