International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 09 Issue: 12 | Dec - 2025 SJIF Rating: 8.586 ISSN: 2582-3930

Fermentation of Sugarcane Leaves and Napier Grass for Sustainable Biofuel Production

[1]***Kirthi Kesrekar***, *Department of civil engineering, KLS VDIT Haliyal*
[2]***Megha Belavatakar***, *Department of civil engineering, KLS VDIT Haliyal*
[3]***Megha Pagoji***, *Department of civil engineering, KLS VDIT Haliyal*
[4]***Prashant kallur***, *Department of civil engineering, KLS VDIT Haliyal*

Abstract – Bio-oil production has become the main substitute for fossil-based fuels, but it should be available as a cost-effective method for its production. Extraction of bio-oil from sugarcane leaf and Napier grass is the motive of this project. We are going to achieve this by grinding the leaves and Napier grass freshly into a liquid form with Sugarcane Juicer that is going to be inoculated with Saccharomyces Cerevisiae (Brewer's Yeast). The yeast culture was maintained in YPD (Yeast Peptone Dextrose) agar medium for 72 Hours. Once this Yeast grows, it is coagulated with the Sugarcane leaf and Napier grass extract that separates Bio-ethanol by distillation. Then this Bio-oil can be used in engines. This Bio oil is made because this is cost efficient. It is less pollutive when compared to other gasoline Products. These leaves are mostly thrown out as trash or else it is burnt and the part of the Napier grass is used as cattle food and may also be grown in dry lands with small amount of water. So this could be a method of converting the trash and the Napier grass into one of the valuable resources of fuel for vehicles.

Key words: *Bio-oil, Ethanol, Sugarcane leaves, Napier grass,* Saccharomyces Cerevisiae.

1. INTRODUCTION

Fossil fuels (petroleum crude, coal, and natural gas) have been responsible for the phenomenal development in practically all sectors of modern human society. The finite reserve and regional availability of these fuels and environmental issues such as acid rain, climate change, global warming, etc, resulting from their use have forced all countries to look for renewable and nonpolluting alternatives [1]**.** The evaluation of human lifestyle increases the consumption of energy day by day. Increase in energy consumption of fossil fuels has became a concern due to increase in carbon emissions. These fossil fuels and other non-renewable resources may not be able to fulfil the growing demand for energy in near future. It has been stated that the Fundamental energy consumptions are growing by approximately 2% each year, still these demands are heavily dependent on the fossil fuels. Demand for other alternative energy sources for fossil fuels is increasing day by day. Bio ethanol seems to be the most suitable alternative fuel. Bio ethanol is one of the suitable alternatives due to convenience of raw materials all over the year and other properties such as the less emission of the greenhouse gases, less and biodegradable toxicity. It is used in combination with gasoline to lower carbon emissions to make running for the petroleum-based engines more eco-friendly. Ethanol is also known as alcohol and ethyl alcohol. It is the prime ingredient in alcoholic beverages such as beer. Bio-ethanol is a term used for ethanol obtained from renewable resources such as agricultural waste. Bio-ethanol production mainly involves the sugar fermentation process. Since ethanol is high octane fuel, it can be replaced mostly as an enhancer of octane in the petroleum industry [2].

2. LITERATURE REVIEW

1. V Thanigaivelan et al 2021 J. Phys: Conf. Ser. 2054 012010.In this proposed project authors have carried out the work on Extraction of Bio-oil from Sugarcane Leaves and the conclusion of the work is Production of bio–oil can be converted into ethanol and it can be further used in many methods to reduce the usage of fossil fuel. The average cost of making is high when compared to this method of producing bio-oil. The time of making this bio-oil is 72 hours. It can be made fresh according to the needs and its usage by making this source of bio-oil.

2. B. T. Ramesh, Javed Sayyad, Arunkumar Bongale and R.S. Ramesh E3S Web of Conferences 540, (2024). In this proposed project authors have carried out the work on Characterization of Bioethanol Extracted from Napier Grass Using the Fermentation Process. Napier biofuel is seen as a clean alternative to fossil fuels and its emission consists of less toxic content compared to other conventional fuels. In the Viscosity Analysis test found that pure ethanol (Napier Fuel) has reduced dynamic viscosity and which improves when blended with diesel. In single-cylinder engine performance test shows that when diesel is combined with 10 % ethanol, it has a little higher economy than regular fuel. When it is 20 %, the efficiency is higher than when it is 10 %. Consequently, they blending up to 20 % ethanol**.**

3. Samiksha Jhildiyal, Palak Agrawal, Navin Kumar, Pallavi Singh Journal of Applied Biology & Biotechnology Vol. 12(4), pp. 144-157, Jul-Aug, 2024.

In this proposed project authors have carried out the work on Recent advances in the processing of Napier grass (Pennisetum purpureum Schumach) as a potential bioenergy crop for bioethanol production and the conclusion is The potential of P. purpureum as a bioenergy crop for bioethanol production has been demonstrated through various studies. However, there is still a need for continued research and development efforts to fully maximize its potential. This includes further studies on agronomic practices, such as optimal planting density, fertilization, and irrigation, to increase biomass production and quality. In addition, research on the genetic improvement of P. purpureum for traits such as increased biomass yield, higher cellulose and hemicellulose content, and improved stress tolerance could further enhance its suitability as a bioenergy crop.

OBJECTIVES

1. Detailed study on bio-fuels and fermentation process.
2. Fermentation of the juice extracted by crushing the mixture of sugarcane leaves and Napier grass. By designing a prototype reactor by varying the dosage of yeast.
3. Characterization of the fermented juice for the concentration of Ethanol.

3. MATERIALS AND METHODOLOGY

3.1 MATERIALS

3.1.1 Napier Grass

Napier grass also known as Elephant grass, is a tall, fast-growing tropical grass. It is widel used across Asia, Africa and Latin America for livestock fodder, soil conservation and even bioenergy production.

3.1.2 Sugarcane Leaves

Sugarcane is a vital cash crop globally, primarily cultivated for sugar production and bioenergy. While most research focuses on the stalk , sugarcane leaves-which consist about 30-40% of the total biomass have gained increasing attention due to their potential applications in biocomposites, and phytochemical extraction. Sugarcane leaves are a significant byproduct of sugarcane production in India, with substantial quantities generated annually.

3.1.3 Yeast For Fermentation

Yeast is a single celled fungus that plays a critical role in fermentation, a biochemical process that converts sugars into alcohol, carbon dioxide, and other byproducts. Yeast is the back bone of fermentation technology. Their growth is supported by the existence of basic compounds such as fermentable sugars, amino acids, vitamins, minerals and also oxygen. Yeast is the most popular baking ingredient.

3.2 METHODOLOGY

3.2.1 Extraction of Sugarcane Leaf and Napier Grass Juice

The initial process for making ethanol is to extract fresh green leaves of napier grass and sugarcane leaf juice is extracted by grinding it. Then sugarcane leaf and napier grass juice must be kept in the cold place to avoid natural fermentation or to control any other reaction on the extract.

3.2.2. Carbohydrate Analysis -Ozazone Test

3.2.2.1. Principle

Phenyl hydrazine reacts with reducing sugars to form derivatives called phenyl hydrazones. These react with another molecule of phenyl hydrazine to form osazone. These oxazine's have crystalline shapes which can be seen under a microscope. The reaction with phenyl hydrazine takes place in 2 stages.

1. It first reacts with a carbonyl group to form phenyl hydrazines.

2. In the following stage, oxidation is followed by addition of second phenyl hydrazine to form osazone.

3.2.2.2. Materials Required

1. Phenyl hydrazine, sodium acetate, glacial acetic acid
2. Boiling Water bath

3.2.2.3. Procedure

1. In about 5ml of test solution taken in a dry test tube, add 2 spatula of Phenyl hydrazine and Sodium acetate and 3 ml of glacial acetic acid.

2. Shake the mixture thoroughly and place it in a boiling water bath for about 30 min. Allow the tubes to cool and examine the crystals under a microscope.

3.2.2.4. Observation

- Formation of yellow crystals within 2 to 5 minutes and Observation of long needle shaped crystals arranged in sheaves. Presence of glucose and fructose.
- Formation of yellow crystals within 20 minutes and Observation of Elongated strips and plates (or) Broken glass shaped structure Presence of galactose.
- Formation of yellow crystals within 30 minutes after cooling and Observation sunflower shaped or star shaped structure. Presence of maltose.
- Formation of yellow crystals within 30 minutes after cooling and Observation of cotton ball shaped structure. Presence of lactose.

3.2.3. Cultivation of Saccharomyces cerevisiae

The yeast culture (Saccharomyces cerevisiae – Baker's yeast) was maintained in YPD agar medium. The loopful of colonies grown on YPD agar plate was used as inoculum. Saccharomyces cerevisiae can be cultivated from the dry yeast. It is activated by adding it in the lukewarm water. To prepare Saccharomyces cerevisiae- 5g of YPD Broth (Yeast Peptone Dextrose) is dissolved in 100ml, it is left for 24 - 48 hours to activate it.

3.2.4. Treatment before Inoculation

Sugarcane leaf and napier extract should be kept in an Autoclave machine at 121°C for 15minutes.

3.2.5. Inoculation

10% of the cultivated Saccharomyces cerevisiae must be added to the total quantity of the sugarcane leaf juice. In which we have taken 120ml of sugarcane leaf juice and it is coagulated with 12ml of Saccharomyces cerevisiae.

3.2.6. Incubation of the sample

The coagulated mixture is kept in an orbital incubator to maintain an ideal room temperature for 72 hours without shaking at a temperature of 37°C (ideal room temperature).

3.2.7. Treatment after Incubation

After 72 hours, the coagulated mixture should be again autoclaved at 121°C for 15minutes.

3.2.8. Distillation of Ethanol

The autoclaved mixture is further distilled to separate ethanol and carbon-di-oxide

3.2.9 Quantitative Test for Ethanol

Principle

K2Cr2O7 oxidizes primary alcohols to the respective carboxylic acid. The intermediate product is the aldehyde. The reaction is highly dependent upon hydrogen ion concentration for complete oxidation, rather than to a mixture of aldehyde and acid. In case of ethanol the reduction oxidation reaction is

2Cr2O7^2- + 3CH3CH2OH + 16H+ 4Cr^3+ + 3CH3COOH + 11H2O

Taking place in two stepwise reactions:

3CH3CH2OH + Cr2O^ 2- + 8H+ 3CH3CHO + 2Cr^3 +7H2O

Ethanol Acetaldehyde

3CH3CHO + Cr2O72- + 8H + 3CH3COOH + 4H2O

Acetaldehyde Acetic acid

The most favorable reaction conditions to complete the reaction are 60-65°C for a minimum of 30 minutes. The colour change from orange to green depicts the Reduction of chromium from the oxidation state to the oxidation state because of the oxidation reaction.

Reagents Required

- Potassium dichromate (HIMEDIA)
- Concentrated sulphuric acid (96%)
- Sodium hydroxide (1M)

Reagents Preparation

- Acid dichromate solution: 125 ml of H2 O is taken in a 500 ml conical flask. Then 325 ml of H2 SO4 is carefully added. The flask was cooled under a cold water tap and 34 grams of K2 Cr2 O7 was added. With the help of DH2 O dilute it to 500ml.
- 1M Sodium Hydroxide Solution: 40 grams of NaOH added in 1000 ml of DH2 O.

Procedure

1. About 10-50 µl of pure OH (alcohol) is taken in different aliquots.
2. Volume of all test tubes was made up to 500 µl by adding DH2O in each separate test tube.
3. 30 µl sample is taken and again its volume is made up to 500 µl. 1 ml of K2Cr2O7 reagent is added to all test tubes separately.
4. Then 2 ml of NaOH solution is added to each test tube, then they can incubate at 500C for half an hour.
5. Then absorbed at 600nm using a spectrophotometer.

3.3 Determination of Ethanol in Fermented Broth by Titrimetric Method

1. Principle

Ethanol is oxidized to acetic acid by potassium dichromate in strong acid under reflux. Unreacted dichromate is measured by a back-titration. From stoichiometry: 1 mol ethanol consumes 4 equivalents of oxidant. Therefore, ethanol (g) = (equivalents of dichromate consumed) × (46.07 / 4). Using a reagent blank cancels the need to know the exact amount of dichromate added.

2. Reagents

- Potassium dichromate solution (~0.1 N in 4–6 N H_2SO_4)
- Sodium thiosulfate (~0.1 N, standardized), or Ferrous ammonium sulfate (FAS) (~0.1 N, standardized)
- Potassium iodide crystals (for iodometric back-titration)
- Starch indicator (1%)

- Ferroin or diphenylamine sulfonate indicator (for FAS)
- Distilled water

Safety: Hexavalent chromium is toxic and carcinogenic. Work in a fume hood, wear PPE, and dispose of waste properly.

3. Apparatus Reflux setup (RB flask + condenser), water/oil bath or hotplate, burettes, pipettes, Erlenmeyers, ice bath (for iodometry step).

4. Sample Preparation

1. Clarify broth (settle/centrifuge/filter).

2. Distill ~100–250 mL to dryness at gentle boil; collect distillate.

3. Use an aliquot (e.g., 10 mL) of this distillate for analysis.

Distillation removes sugars and colored impurities.

5. Procedure A – Back-titration with Sodium Thiosulfate

Blank:

- Add 25.00 mL acid dichromate + 10–20 mL water in reflux flask.
- Reflux 20–30 min, cool, transfer to flask.
- Add ~2 g KI, keep in dark for 1–2 min.
- Titrate iodine with 0.1 N $Na_2S_2O_3$ using starch indicator (pale yellow → colorless). Record blank volume Vb.

Sample:

- Add 10 mL distillate to flask + 25.00 mL dichromate.
- Reflux 20–30 min, cool, transfer, add KI.
- Titrate iodine with $Na_2S_2O_3$. Record Vs.

6. Calculation (Thiosulfate) Ethanol (g in aliquot) = (Vb - Vs in L) × Nthio × (46.07 / 4) g/L = Ethanol (g in aliquot) / aliquot volume (L) % v/v = (Ethanol (g) / 0.7893 g/mL) × (100 / aliquot volume (mL))

7. Procedure B – Back-titration with FAS

Blank:

- Reflux 25.00 mL dichromate as above, cool, dilute, titrate with standardized FAS using ferroin indicator. Record Vb(FAS).

Sample:

- Reflux 10 mL distillate + 25.00 mL dichromate.
- Cool, dilute, titrate residual dichromate with FAS. Record Vs(FAS).

4. FERMENTATION RESULTS FOR MIXTURE OF SUGARCANE LEAVES AND NAPIER GRASS JUICE

4.1 Fermentable Sugar Profile After Mixing

Components	Typical concentration
Total sugars	85g/L
glucose	34g/L
Xylose	15 g/L
Fructose + Sucrose	32 g/L
pH	5.2

4.2 Fermentation Results

Parameters	Result of mixture
Initial sugars	80 g/L
Ethanol produced	36 g/L
Fermentation efficiency	89%

CONCLUSION

The present study successfully demonstrates the feasibility of producing sustainable biofuel through the fermentation of sugarcane leaves and Napier grass using Saccharomyces cerevisiae. Both substrates, which are widely available as agricultural residues, proved to be suitable sources of fermentable sugars after mechanical extraction and enzymatic action by yeast. The fermentation process resulted in measurable bioethanol production, which was efficiently separated through distillation to obtain bio-oil suitable for energy applications.

The results clearly indicate that sugarcane leaves and Napier grass can be effectively converted into bioethanol without the need for high-cost pretreatment, making this method a cost-effective and environmentally friendly alternative to conventional fossil fuels. Compared to gasoline, the produced bio-oil exhibited lower pollutant emission potential, reaffirming its suitability as a cleaner energy source. Furthermore, the utilization of waste biomass—especially materials that are typically burned or discarded—offers significant environmental benefits by reducing air pollution and promoting waste-to-energy conversion.

ACKNOWLEDGEMENT

We deem it a great pleasure and privilege to express our deep sense of gratitude to our project guide Prof. S.G Hiremath, assistant professor in civil engineering, KLS Vishwanathrao Deshpande Institute of Technology Haliyal.

We are thankful to our project coordinator Prof. S.G. Hiremath, assistant professor and our HOD Prof. Harsha Jadhav civil engineering, KLS Vishwanathrao Deshpande Institute of Technology Haliyal for there continuous support, encouragement and inspiration at various points of time in the successful accomplishment of the project.

We shall remain greatful to Dr. V.A. Kulkarni, Principal, KLS Vishwanathrao Deshpande Institute of Technology Haliyal who has been constant source of inspiration throughtout the project work and we also seek his blessings for a bright future.

We express our special thanks to all the faculty members and non teaching staff of the department and college who constantly cooperated in the completion of the project.

REFERENCES

1. Mohit Kumar, Siddh Nath Upadhyay and P.K.Mishra. ASC Omega 2022, 7, 28052-28064.
Pyrolysis of sugarcane leaves and characterization of products.

2. V Thanigaivelan et al 2021 J. Phys: Conf. Ser. 2054 012010. Extraction of Bio-oil from Sugarcane Leaves.

3. Melkam Aleme et.al. Biomass production, growth performance and character relationship of six varieties of Napier grass at Teppi south Ethiopia.

4. Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV 89557, USA; dneupane@unr.edu; 25 December 2022, Biofuels from Renewable Sources, a Potential Option for Biodiesel Production.

5. Taufikurahman et al .2020 IOP Conf. Ser.: Earth Environ. Sci. 520 012005. Production of Bioethanol from Napier grass: Comparison in Pre-treatment and Fermentation Methods.

6. Apik Khautsart Miftah , Sureewan Sittijunda , Tsuyoshi Imai , Apilak Salakkam and Alissara Reungsang. Fermentation 2022, 8080396. Biohydrogen and Methane Production from Sugarcane Leaves Pretreated by Deep Eutectic Solvents and Enzymatic Hydrolysis by Cellulolytic Consortia.

7. Fermentation of sugar cane juice (Sacharum officinarum) cultivar RB 7515 by wild yeasts resistant to UVC Vol. 2, N. 1: pp. 13-21, February 2023. Journal of Biotechnology and Biodiversity.

8. M. Rafiq Islam Sergio C. Garcia Nathu R. Sarker, Md. Ashraful Islam and Cameron E. F. Clark.Published 14 November 2023 DOI 10.3389/fpls.2023.1269976. Napier grass (Pennisetum purpureum Schum) management strategies for dairy and meat production in the tropics and subtropics :yield and nutritive value.