Extraction of Bio-oil from Sugarcane Leaves

V Thanigaivelan1,*, T K Kishore Kumar2, G Hari Prasad3, K Daniel Jeysingh4, R Mohit5

1 Assistant Professor, Department of Mechanical Engineering, SRM Institute of Science and Technology, Ramapuram Campus, Chennai.

2,3,4,5 UG Students, Department of Mechanical Engineering, SRM Institute of Science and Technology, Ramapuram, Chennai.

*Corresponding author email: thanigailav@gmail.com

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 is the motive of this project. We are going to achieve this by grinding the leaves 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 extract that separates Bioethanol 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, so this could be a method of changing those trash into one of the Valuable resources of fuel for Heavy vehicles.

Keywords: Bio-oil, Ethanol, Sugarcane Leaf, Saccharomyces Cerevisiae

1. Introduction

The evolution of human lifestyle increases the consumption of energy day by day. Increase in energy consumption of fossil fuels has become a concern due to increase in carbon emissions. These fossil fuels and other non-renewable resources may not be able to fulfill 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. British Petroleum's annual report on ‘The proven global oil reserves’ declare that as of the end of the year 2016, Earth has almost 239.4 million tons of crude fossil fuels, which would last only for 53.38 years at current rates of extraction. The recent advancement of biofuels has been driven by three key global challenges: Security for energy, a big Economic development, Climate change. It has been reported that the Biofuels production increased by 0.9% in 2015. (British Petroleum, 2015).

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. Bioethanol is a term used for ethanol obtained from renewable resources such as agricultural waste. Bioethanol 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. The sugar source comes from fuel or energy crops such as sugarcane, molasses, wheat, barley, and corn etc. Currently Brazil and the US have the most bioenergy plants producing bioethanol. As ethanol dissolves in H2O and other organic compounds easily, it is an ingredient in personal care, beauty products, paints and varnishes to the fuel. The multifarious applications of bioethanol have left the ethanol industry flourishing.

The best way to get good results is by mixing out a tiny portion of a volatile fuel like petrol. The mixture of OH with petrol or diesel will be used. The most conventional blends are (by volume) as follows:

- E5G to E26G (5-26% OH, 95-74% petrol)
- E85G (85% OH, 15% petrol)
- E15D (15% OH, 85% diesel)
- E95D (95% OH, 5% water, with ignition improver).

Saccharomyces cerevisiae (Baker’s yeast) is a single-celled eukaryote. It ferments sugar into carbon dioxide and alcohol. Saccharomyces cerevisiae, the most employed yeast for the baking, brewing industries and for the production of ethanol. The demand for ethanol has been increasing rapidly because the government of India has decided to add more than 10% of ethanol to petrol. Sugarcane leaves are mostly thrown out as trash or else it is burnt, so this could be a method of changing that trash into one of the valuable resources of fuel. The preparation cost of biofuel made from sugarcane leaves is very low compared to other biofuel made from animal fat and plant oils. Farmers could be getting an additional income by converting these leaves. This is also a way to reduce dependency on fossil fuel and greenhouse gases (GHG). Sugarcane leaves are a potentially renewable resource that may be used to produce bioethanol.

2. Materials and Methods

2.1. Extraction of Sugarcane Leaf Juice

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

2.2. Carbohydrate Analysis - Ozonone Test

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.

2.2.2. Materials Required

- Phenyl hydrazine, sodium acetate, glacial acetic acid
- Boiling Water bath

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.

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 sunflowershaped or star shaped structure. Presence of maltose.
• Formation of yellow crystals within 30 minutes after cooling and Observation of cotton ballshaped structure. Presence of lactose.

Figure 1. Long needle shape crystal

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) isdissolved in 100ml, it is left for 24 - 48 hours to activate it.

2.4. Treatment before Inoculation
Sugarcane leaf extract should be kept in an Autoclave machine at 121°C for 15minutes.

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.

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).

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

2.8. Distillation of Ethanol
The autoclaved mixture is further distilled to separate ethanol and carbon-di-oxide.

2.9. Qualitative Test for Ethanol
2.9.1. Reagents Required
1. Potassium iodide (0.2 M)
2. Iodine (0.5 M)
3. Sodium Hydroxide (1 M)
4. Methanol
2.9.2. Reagents Preparation
1. 0.2 M Potassium iodide: 0.664g of Potassium iodide was dissolved in 20 mL of water.
2. 0.5 M Iodine: 4 g of Potassium iodide and 1.27 g of Iodine was dissolved in 10 mL of water.
3. 1M NaOH: 0.4 g of NaOH was dissolved in 10 mL of water.

2.9.3. Procedure
- 10 drops of methanol, ethanol, were taken in separate test tubes.
- 10 drops of water were taken in a separate test tube as a control.
- To each test tube 25 drops of Iodine solution was added and then 10 drops of Sodium hydroxide was added.
- The test tubes were swirled and then it was incubated at room temperature.

2.10. Quantitative Test for Ethanol
2.10.1. 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

2Cr2O72- + 3CH3CH2OH + 16H+ → 4Cr3+ + 3CH3COOH + 11H2O

Taking place in two stepwise reactions:
3CH3CH2OH + Cr2O 2- +8H+ → 3CH3CHO + 2Cr3++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 [VI] oxidation state to the [III] oxidation state because of the oxidation reaction.

2.10.2. Reagents Required
- Potassium dichromate (HIMEDIA)
- Concentrated sulphuric acid (96%)
- Sodium hydroxide (1M)

2.10.3. Reagents Preparation
- Acid dichromate solution:
  125 ml of H2O is taken in a 500 ml conical flask. Then 325 ml of H2SO4 is carefully added. The flask was cooled under a cold water tap and 34 grams of K2Cr2O7 was added. With the help of DH2O dilute it to 500ml.
- 1M Sodium Hydroxide Solution:
  40 grams of NaOH added in 1000 ml of DH2O.

2.10.4. 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 500°C for half an hour.
5. Then absorbed at 600nm using a spectrophotometer.

3. Result and Discussion

3.1. Colony count of *Saccharomyces cerevisiae*

The serial dilution was done from inoculums from $10^{-1}$ to $10^{-9}$ and kept for incubation for 48 hours. Optimum growth was observed in plates containing $10^{-5}$ and $10^{-6}$ dilutions.

![Figure 2. Colony count of *Saccharomyces cerevisiae*](image)

The colonies obtained at
- $10^{-5} = 340 \times 10^6$ CFU/mL
- $10^{-6} = 180 \times 10^7$ CFU/mL

3.2. Ethanol Confirmation

In this study, the ethanol extracted from the yeast fermentation of *Saccharomyces cerevisiae*. Ester, Litmus, and Iodoform Test confirms the presence of ethanol. Figure 3 shows the cloudy white precipitate in the sample. This infers the presence of ethanol.

| Time | Ester | Litmus | Iodoform |
|------|-------|--------|----------|
| 48   | +     | +      | +        |
| 72   | +     | +      | +        |
| 96   | +     | +      | +        |
| 120  | +     | +      | +        |
Figure 3. Test for Ethanol

Table 2. Effect of time

| Time (hrs) | Concentration of Ethanol |
|-----------|--------------------------|
| 48        | 1.44                     |
| 72        | 1.59                     |
| 96        | 1                        |
| 120       | 0.6                      |

From the results it is observed that the concentration of the extracted ethanol was maximum at 72hrs of fermentation.

3.3. Ethanol Estimation

3.3.1. Standard plot for Ethanol
Ethanol is estimated by reacting water and ethanol at different proportions with Acidified Potassium Dichromate and NaOH. Then standard estimation was done by measuring the Optical Density (OD) after incubation at 50°C for 30 min. The table 3 shows the standard ethanol estimation. The graph in figure 4 is plotted by the estimation of Optical Density (OD).

Table 3. Standard plot for Ethanol estimation

| Reagents                              | Blank | S1  | S2  | S3  |
|---------------------------------------|-------|-----|-----|-----|
| Volume of Ethanol (99%) (uml)         | -     | 10  | 50  | 100 |
| Volume of water (uml)                 | 500   | 490 | 450 | 400 |
| Concentration of Ethanol (g/l)        | -     | 1.5 | 7.9 | 15.7|
| Acidified Potassium Dichromate (mL)   | 1     | 1   | 1   | 1   |
| NaOH (mL)                             | 2     | 2   | 2   | 2   |
| Incubated at 50 °C for 30 min         |       |     |     |     |
| OD was measured at 580 nm             | 0     | 0.2 | 0.75| 1.72|
4. Conclusion

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. Farmers and corporate people can make profit on both sides. Farmers can sell their leaves for a price that can be an additional source of income and corporate people can make these types of products by investing less.

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Figure 4. Standard plot for Ethanol estimation