Value Added Bioethanol Fuel from Waste Decayed Manilkara Zapota Fruit

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Abstract. In this research work, it was attempted to give value addition to the waste decayed Manilkara Zapota fruit by producing bioethanol from it. Manilkara Zapota fruit wastes were taken as a substrate for the microorganism (Saccharomyces cerevisiae) to grow under a controlled environment in order to facilitate the fermentation process. The temperature of fermentation process was maintained at 37°C with the help of biological oxygen demand incubator for 72 hrs. Once the fermentation process was over the bio-ethanol was extracted by distillation process at the temperature of 72°C. The purity of the produced ethanol was identified using infrared spectroscopy and gas chromatography mass spectrometry. The physicochemical properties such as density, pH, molecular weight, octane number, flash point, freezing point and autoignition temperature were measured. The yield of bio-ethanol from decayed Manilkara Zapota fruits was found to be 10.45% (w/v). The GC-MS results infer that the purity of ethanol obtained in the sample is 99.09%.

Keywords: Manilkara zapota, Bio-ethanol, Fermentation, Saccharomyces Cerevisiae, Distillation.

1. Introduction

The ethanol, a colourless, flammable and volatile liquid, popularly known as ethyl alcohol or cologne spirit is a constitutional isomer of dimethyl ether made by a carbon of a methyl group attached with the carbon of a methylene group which is attached with the oxygen of a hydroxyl group. It has many practical applications such as drug, recreational drink, disinfectant, antiseptic and chemical solvent in industries. It is also used as alternative for fossil fuels, fuel for electricity production, direct fuel cell applications and refrigeration application. It is produced by two methods (i) acid catalyzed hydration of ethylene (ii) fermentation of sugar. The ethanol produced through second method is known as bioethanol.

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India, the fourth largest ethanol producer in the world, looking for bioethanol production from low cost feed stocks as 50 to 60% of production cost of the bioethanol is accounted towards the cost of feed stock. In this regard the current research focus is on the production of bioethanol through inexpensive feedstocks such as lingocellulosic and agro-food wastes. The decayed fruit wastes are such feedstocks best suited for bioethanol production.

Manilkara Zapota, one of the largest harvested fruits in India, contains more sweetener in its pulp. This pulpy fruit is more prone to spoilage due to its nature. This wastage may happen during the time of harvesting, transportation and storing of fruits in stockyard. A statistics reported that 30 to 40% of the fruits harvested in India may get damaged and become waste and thrown in to garbage yard. This decayed fruit waste thrown in to garbage yard are underutilized and readily available agricultural waste can be used as potential growth medium for yeast strain (Brooks, 2008; Essien et al., 2005; Hueth and Melkonyan, 2004; Hammond et al., 1996), and could serve as a good substrate for ethanol production.

The main objective of this research article is utilization of valueless decayed Manilkara zapota fruits as substrate to produce value added bioethanol through fermentation process using Saccharomyces cerevisiae (baker’s yeast) followed by distillation process. Characterization of the produced bioethanol for quality checking is also reported in this article.

2. Materials and Methodology

2.1. Preparation of substrate and inoculum for fermentation process

Decayed Manilkara Zapota fruits were collected from the local fruit market in and around Chennai. Collected fruits were washed using clean water to remove the dust and other pollutants. After washing the fruits in clean water, the fruits were cleaned in 5% Potassium Permanganate solution (KmNO4). Potassium Permanganate acts as an antiseptic. The purpose of treating the Manilkara Zapota fruits in KmNO4 solution is to remove the infections already caused by any other micro-organism. Then the fruits were rinsed and cleaned in distilled water in order to remove Potassium Permanganate. This step helps in avoiding unnecessary reactions taking place between Potassium Permanganate and saccharomyces cerevisiae. Potassium Permanganate will give adverse results if it reacts with yeast. Saccharomyces cerevisiae will be killed by KmNO4 solution as it is an antiseptic. Due to this the fruits were thoroughly washed with distilled water before adding yeast. The seeds from the cleaned, disinfected Manilkara Zapota fruits were removed. The seedless fruits were then mashed using a juicer. The pulpy mass is the substrate as shown in figure 1.
Inoculum is prepared by mixing 1g of urea, 50g of sucrose and 10g of Saccharomyces cerevisiae (Yeast) with warm water in a separate beaker. Saccharomyces cerevisiae is able to perform both aerobic and anaerobic respiration. It uses two enzymes namely Invertase and maltase in order to break down the disaccharides and polysaccharides into monosaccharides. An enzyme zymase secreted by saccharomyces cerevisiae is used to catalyse the conversion of monosaccharide into ethanol. 200 g (± 0.1) of prepared substrate is placed in a 1.5 litres conical flask and prepared amount of inoculum is transferred to the flask and required amount of distilled water is added to make the final volume to 1000ml. The sample is kept undisturbed in the incubator at 37°C. During the incubation the specific gravity of the sample is measured at the end of every 12 hours using hydrometer. When the specific gravity reaches the steady value, it is the indication of end of fermentation process. The photographic view of the fermentation process in the incubator during different days is shown in figure 2.

2.2. Ethanol extraction by distillation process
Once the fermentation process is over, the conical flask is removed from the incubator and the product is taken for distillation. Distillation is the process of separating the component substances from a liquid mixture by selective evaporation and condensation processes. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture.
Selective evaporation is achieved by setting the temperature of heating element to the boiling point of ethanol. The distillation unit used in this study consists of temperature controllable heating mantle, three necked round bottom flask, water cooled condenser and distillate collecting flask as shown in figure 3. The contents of the conical flask are transferred to a round bottom flask and kept in a heating mantle. The outlet of the round bottom flask is connected to a condenser where the ethanol condenses and the condensate is collected.

2.3. Infrared spectroscopy system
Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the transition energy of the bond or group that vibrates. The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond or collection of bonds, absorption occurs. Examination of the transmitted light reveals how much energy was absorbed at each frequency (or wavelength). This measurement can be achieved by scanning the wavelength range using a monochromator.

2.4. Gas chromatograph system
The amount of bio-ethanol and its purity were measured using a gas chromatograph instrument facilitated with mass spectrum. Chromatography is the study of the separation of mixtures of any chemical or oil or fat, and is often used to identify unknown components in a mixture. In chromatography, the components in a mixture move along a stationary phase. Each component in a mixture moves at a rate based on its own characteristics and properties. Mixture to be separated is passed into the mobile phase through the stationary phase to achieve separation of the individual component in a mixture using the rate of migration. The mobile phase flow rate (RF) across the separation medium is measured in ml/min or μl/min. A mass spectrometric detector (MSD) attached gas chromatography is known as GC-MS. It is a powerful tool to identify the components in a mixture. In GC-MS, a compound in a mixture is first converted into ionic fragments, and then the total number of ions are detected and plotted against time.

In this study, Perkin-Elmer Clarus 500 Auto System XL Gas Chromatograph equipped with Elite series PE - 5 capillary column of dimension 30m x 0.25mm x 1μm is used. The oven temperature was initially held at 100°C for 10min, later increased to 200°C at the rate of 10°C/min and then held for 10min. The injector, source and the transfer line temperatures were 220°C, 200°C and 200°C respectively. DB-1 (100% dimethylpolysiloxane) column with Helium as carrier gas at a flow rate of 1 ml/min was used. The equipment has a mass spectrometer of Turbo EI Ionization source of 70 eV electron energy and ion energy of 1.5 V. The samples were injected in split mode as 10:1. Mass
spectral scan range was set at 45-450 (m/z). Ethanol identification was carried out using NIST Ver.2.1 MS data library.

3. Results and Discussions

3.1. Identification of completion of fermentation process
Measure of specific gravity of the fermentation sample is one way of identifying the completion of fermentation process. The specific gravity of the fermentable product before the start of the fermentation process would be higher and when fermentation starts, the value of specific gravity will keep on reducing with respect to the rate of fermentation. This decrease in specific gravity is a clear indication of yeast fermenting the sugar into ethanol. Once the fermentation process is completed the specific gravity would reach a final value and remains constant. The variation of specific gravity of the sample with respect to time is measured and plotted as shown in figure 4. Initially the value of specific gravity of the sample is 1.08 and at the end of the 4th day its value reached 0.978 and remained stable. This stabilized value of specific gravity of the sample ensures the completion of the fermentation process.

![Figure 4. Variation of Specific Gravity with Time.](image)

3.2. Infrared Spectroscopy Results
The IR spectroscopy result of the produced bio-ethanol sample is shown in figure 5. The graph clearly depicts the presence of OH group (Alcohol functional group) in the produced sample. The peak at 3358.43 cm⁻¹ is the evidence of the OH group. As the result of IR spectroscopy will not quantify the amount of ethanol produced, gas chromatography test was carried out using GC-MS system to ascertain purity and quantity of ethanol production.
3.3. Gas Chromatograph Results

The gas chromatograph sample of the produced bio-ethanol is shown in figure 6. The peak value of the chromatograph sample and its comparison with NIST library sample peak for ethanol are presented in figure 7. From the chromatograph analysis the % of bio-ethanol in the sample is calculated using the following formula. Percentage of Ethanol (%) = Individual peak area / total peak area *100, from the calculation it was found that 99.09% of pure bio-ethanol was present in the sample. The yield of bio-ethanol from decayed Manilkara Zapota fruits was found to be 10.45% (w/v).
3.4. Physicochemical properties of Bio-ethanol

Major properties of Bio-ethanol namely density, molecular weight, auto-ignition temperature, heating value, freezing point, flash point, stoichiometric A/F ratio, and octane number were estimated as per ASTM standards and compared with laboratory ethanol and reported in Table 1.

| Sl.No. | Parameters                   | Bioethanol | Ethanol |
|--------|------------------------------|------------|---------|
| 1      | Density (kg/m³)              | 978        | 789     |
| 2      | pH                           | 5.5        | 7.33    |
| 3      | Molecular weight (g/mol)     | 46         | 46.07   |
| 4      | Octane number                | 108        | 113     |
| 5      | Auto-ignition temp. (°C)     | 425        | 365     |
| 6      | Stoichiometric A/F ratio     | 9.00       | 9.00    |
| 7      | Flash point (°C)             | 23         | 16.63   |
| 8      | Heating value (MJ/kg)        | 26.9       | 27.3    |
| 9      | Freezing point (°C)          | -40        | -114    |

4. Conclusion

Waste decayed Manilkara zapota fruit pulp has been considered for bioethanol production using saccharomyces cerevisiae yeast through fermentation process. The following conclusions were arrived based on the outcome of the research work.

- Decayed Manilkara zapota fruits can be one of the best substrate for bio-ethanol production.
200g of Manilkara Zapota was found to produce approximately 21ml of bio-ethanol when 10gm of Saccharomyces cerevisiae (Baker’s Yeast), 50gm Sucrose and 1gm urea were added for fermentation process.

The yield of bio-ethanol from decayed Manilkara Zapota fruits was found to be 10.45% (w/v).

The GC-MS results infer that the purity of ethanol obtained in the sample is 99.09% by volume.

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