Fatty acid ethyl ester from Manilkara zapota seed oil: A completely renewable biofuel for sustainable development

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Research Article

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Abstract

This article reports the deliverables of the experimental study on the production of a completely renewable biofuel from Manilkara zapota fruit. It was attempted to produce fatty acid ethyl ester from Manilkara zapota seed oil using bioethanol synthesized from decayed Manilkara zapota fruit. Bioethanol was produced through fermentation of decayed Manilkara zapota fruit, waste skin, and pulp with Saccharomyces cerevisiae then extracted by distillation process at the temperature of 72°C. The bioethanol yield was noted as 10.45% (v/w). The purity of the bio-ethanol was identified as 95.09% using infrared spectroscopy and gas chromatography-mass spectrometry. Mechanically extracted Manilkara zapota seed oil was used for ethyl ester production. The molar ratio of bioethanol to oil, the quantity of KOH, and process temperature were optimized for the maximum yield of Manilkara zapota ethyl ester. 9:1 molar ratio of bioethanol to oil, 1.5% (w/w) KOH, and 70°C process temperature were identified as optimized ethanolysis process parameters. The maximum yield of ethyl ester was identified as 93.1%. Physicochemical characteristics of Manilkara zapota oil, bioethanol, and ethyl ester were measured as per the corresponding ASTM standards. It was found that both Manilkara Zapota ethyl ester and bioethanol synthesized from decayed Manilkara zapota fruit could be promising substitutes for fossil diesel and gasoline.

1. Introduction

Biodiesel, a mono-alkyl ester has been produced from many resources by many researchers. The major resources predominantly used for biodiesel production are edible and nonedible plant-based seed oils and some animal fats. Among the above mentioned raw materials, plant-based edible oils have more benefits such as low free fatty acid content, lower viscosity than that of non-edible oils, abundant availability, straightforwardly cultivatable in a short span of time, presence of a high amount of monounsaturated fatty acids and other suitable properties to readily convert into biodiesel (Hulya Karabas 2013; Xuan and Dennis 2011; Kafuku and Mbarawa 2010a). Biodiesel synthesized from edible vegetable oils is generally recognized as first-generation biodiesel. Few first-generation biodiesel resources are coconut oil, peanut oil, sunflower oil, gingelly oil, mustard oil, rice bran oil, and mahua oil. The non-edible vegetable oils are biodiesel resources that belong to the second generation group. They are preferable to edible oils because of the low cost (Melvin et al. 2011; Kafuku and Mbarawa 2010b; Akin et al. 2008).

The popularly used alcohol for biodiesel production is methanol or methyl alcohol. It is highly reactive in the transesterification process. Though methanol is alcohol with lower molecular weight and highly reactive in the conversion of biodiesel, it is toxic and synthesized chemically. It is vulnerable to human lives. Unknowing inhalation causes suffocation problems for human beings. Consumption of 10 ml pure methanol affects optic nerves and causes blindness; consuming beyond that sometimes causes fatal also. Hence ethanol nontoxic alcohol could be a better substitute for methanol in biodiesel production (Brooks 2008; Korkie et al. 2002; Neelakandan and Usharani 2009; Naresh et al. 2007; Mohammad and Keikhosro 2007).
Ethanol is a colorless, flammable, and volatile liquid. It is popularly known as ethyl alcohol. It consists of two single-bonded carbon atoms of the alkyl group and oxygen of a hydroxyl group (Farid et al. 2010; Tiwari et al. 2010; Tiwari et al. 2011; Veeranjaneya and Vijaya 2007; Abul and Nilufa 2019; Sargar et al. 2017). It has many practical applications such as drug, recreational drink, disinfectant, antiseptic, refrigerant, and chemical solvent in industries. It is also used as an alternative to fossil fuels. It is produced by two methods (i) acid-catalyzed hydration of ethylene (ii) fermentation of sugar. The ethanol produced through the second method is known as bioethanol (Jahid et al. 2018; Maziar 2010; Pramanik and Rao 2005; Sanjeev et al. 2004; Suresh et al. 2020).

India, the fourth largest ethanol producer in the world, is looking for bioethanol production from low-cost feedstocks. Around 60% of the production cost of bioethanol is accounted for the cost of feedstock, hence identification of low-cost feedstock for bioethanol production is highly needed (Abdelrahman et al. 2020; Recep 2020; Dawid and Grzegorz 2020). In this regard, the current research focus is on the production of bioethanol through inexpensive feedstock such as lingo cellulosic 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 saccharinity in its pulp. This pulpy fruit is more prone to spoilage due to its nature during the time of harvesting, transportation, and storing in the stockyard. 20 to 25% of the *Manilkara zapota* fruits harvested in India may get damaged; become waste and thrown into garbage yard. Fruit skins and waste pulps from *Manilkara zapota* pulp, jam, and juice industries are also thrown into garbage yard. These decayed fruit waste thrown into the garbage yard are underutilized and can be used as a potential growth medium for yeast strain, which could be a better resource for bio-ethanol production. The seeds from the fruits contain around 20–30% (w/w) oil content could be a potential oil resource for biodiesel production (Sathish Kumar et al. 2018; Sathish Kumar and Sureshkumar 2016; Sathish Kumar et al. 2015).

Through an intensive literature review, it is observed that very little research efforts have been attempted to synthesize biodiesel using bioethanol synthesized from decayed fruits or waste vegetables. Also, no literature contribution has been found on ethyl ester production using zapota seed oil with bioethanol synthesized from *Manilkara zapota* fruit. This experimental research work aimed to study the utilization of valueless decayed *Manilkara zapota* fruit wastes from the cultivation field and pulp industries as a substrate to produce value added bioethanol through the fermentation process using *Saccharomyces cerevisiae* (baker’s yeast) followed by the distillation process. Further, use that bioethanol for ethyl ester production from *Manilkara zapota* seed oil. Characterization of the produced bioethanol and biodiesel for quality checking has been carried out and reported in this article.

**2. Materials And Methodology**

**2.1. Materials**

Decayed fruit wastes were collected from the local fruit market and cultivation fields in and around Chennai, India. Manilkara fruit skins, pulp wastes, and seeds were collected from the pulp, jam, and juice
industries in Andrapradesh state, India. KOH pellets, Potassium Permanganate, urea, sucrose, and *Saccharomyces cerevisiae* were acquired from Pentagon chemicals manufacturer, Chennai, India. The experimental setup used for the transesterification process consists of a magnetic stirrer with a hot plate (Remi, India), a thermometer of 0 to 100°C range, 200 ml flat bottom conical flask with a reflex condenser, and 200 ml separating funnel. The schematic representation of the transesterification apparatus is presented in Fig. 1. A standardized digital balance of 0.001 g accuracy was used for accurate weighing of raw materials. A precise temperature controllable incubator of temperature range 0 to 100°C was used to preserve the fermentation process. The distillation unit used in this study consists of a temperature controllable heating mantle, a three-necked round bottom borosilicate glass flask of 5-liter capacity, a water-cooled condenser, and a distillate collecting flask as shown in Fig. 2.

### 2.2. Preparation of Substrate and Inoculum for Fermentation Process

Collected fruit wastes and skins were washed using clean water to remove the dust and other pollutants then cleaned in 5% potassium permanganate solution (KMnO₄) to remove the infections already caused by any other micro-organism. Then they were rinsed and cleaned in distilled water to remove potassium permanganate to avoid adverse reaction between KMnO₄ and yeast. The seeds from the cleaned disinfected *Manilkara zapota* fruits were removed for oil extraction. The seedless fruits, skins, and collected pulps were then smashed with a juicer to prepare the substrate. The inoculum was prepared by mixing 10 g of *Saccharomyces cerevisiae* (baker's yeast), 50 g of sucrose, and 1 g of urea, with 100 g of warm water in a distinct glass container. 200 g of the prepared substrate was placed in a 1.5 liters conical flask and the measured amount of inoculum was transferred to the flask along with the required amount of distilled water to make the final volume to 1000 ml. The sample was kept undisturbed in the incubator at 37°C. During the incubation, the specific gravity of the product was measured at the end of every 12 hours using a hydrometer. The attainment of a steady value of specific gravity indicates the end of the fermentation process. The photographic views of the fermentation process in the incubator during different days are shown in Fig. 3.

### 2.3. Ethanol Extraction by Distillation Process

Once the fermentation process is over, the conical flask is removed from the incubator and the product is subjected to distillation. The extraction process of a distinct substance from a liquid mixture using selective evaporation followed by condensation is called distillation. The purity of the extracted components using distillation is very high. Selective evaporation is achieved by setting the temperature of the heating element to the boiling point of ethanol. The contents of the conical flask are transferred to a round bottom flask and heated and maintained exactly to a boiling point of ethanol in a heating mantle. A water-cooled condenser with a collecting flask is connected to the outlet of the round bottom flask to condense and collect the evaporated ethanol.

### 2.4. Infrared Spectroscopy Test for Bioethanol

An infrared (IR) spectrometry test is conducted to confirm the presence of ethanol in a distillate product. Each molecule present in the bioethanol absorbs the infrared rays at certain wavelengths based on their structural characteristics. The infrared light beam emitted from the IR source is continuously passed to
the bioethanol sample at different wavelengths to record the IR spectrum. The absorption of the IR beam
occurs when the vibrational frequency of the molecule matches with source IR wavelength. The amount
of energy absorbed at each wavelength of the IR beam is measured by a monochromator.

This research uses the JASCO 6300 model Fourier transform infrared spectrometer. Two NaCl salt plates
are washed using dichloromethane and cyclohexane with full care, without any finger prints on the plates.
In the middle of one plate, a drop of bioethanol sample is deposited and another plate is placed on the
top, then softly squeezed together and rotated slightly using a soft tissue to create a thin film of
bioethanol sample on the plates. The sample plates are then carefully placed in the holder of the sample
chamber in the instrument. The scale is fixed to 4000-400cm$^{-1}$ and the range is fixed to 0-100\%T and
spectrum is recorded. Once the spectrum is recorded the sample plates are cleaned.

2.5. Gas Chromatograph Test for Bioethanol, Raw Oil and Ethyl Ester
The amount of bioethanol produced and its purity was measured using a gas chromatograph instrument
facilitated with the mass spectrum. The chemical composition and components present in any solution
or oil or fat can be identified and quantified by gas chromatograph test. When the sample is injected in a
stationary or source column, each component present in the mixture will move at a different rate based
on their characteristics. The injected sample is passed to the transfer line to achieve separation of the
individual component in a mixture using the rate of migration. The mobile or transfer phase flow rate is
measured in ml/min or µl/min. A mass spectrometric detector (MSD) attached gas chromatography is
known as GC-MS. In GC-MS, a compound in a mixture is first converted into ionic fragments and then the
total number of ions is detected and plotted against time.

In the present investigation, Turbo EI mass spectrometer inbuilt Perkin-Elmer Clarus 500 gas
c Chromatograph instrument is used to identify the chemical composition of raw oil and bioethanol. The
specification of the capillary column is 30m x 0.25mm x 1µm. The carrier gas used in this instrument is
Helium with a flow rate of 1 ml/min. The initial temperature of the oven is set to 100°C and maintained
for 10 minutes to attain stability, later raised to 200°C, and remained constant. The rate of increase in
oven temperature is 10°C/min. The injector, source, and transfer line temperatures are set to 220°C,
200°C, and 200°C respectively. NIST Version 2.1 MS data library is used to identify the components.

2.6. *Manilkara Zapota* Ethyl Ester Production Using Bioethanol

The *Manilkara zapota* seeds were collected and dried for 48 hours in sunlight, shells were removed
manually and oil was extracted using a mechanical expeller. The oil was characterized using ASTM
standards. 100 g of pure *Manilkara zapota* oil was poured into a conical flask and heated up to the
required temperature and maintained constantly. The oil was continuously stirred at 500 rpm using a hot
plate with a magnetic stirrer. The ethoxide solution was prepared separately by thoroughly dissolving the
required quantity of KOH in the required amount of bioethanol. The oil temperature, amount of KOH, and
quantity of bioethanol were fixed with respect to the corresponding experimental conditions. Upon
attaining the required temperature of the oil, the ethoxide solution was gradually transferred into the
conical flask to initiate the transesterification reaction. All the experiments were conducted for 90 minutes.
and then the products were shifted to the separating funnel and kept undisturbed for 24 hours to facilitate the phase separation. The low viscous biodiesel settled on the top was separated and cleaned using distilled water for removing the unreacted chemicals.

2.7. Optimization of Ethanolysis Process Parameters

Three highly influencing ethanolysis process parameters were taken for the optimization namely bioethanol to oil molar ratio, catalyst quantity, and oil temperature. Other transesterification parameters such as reaction time and stirring speed were maintained constant at 90 minutes and 500 rpm respectively throughout the optimization study. By performing a series of experiments with various values of that particular parameter and holding other parameters constant at some arbitrary value, the optimum value of a particular parameter was obtained. The obtained optimal value of a parameter was held constant for the optimization of the other parameters until the optimum value was achieved for that particular parameter. Three replications were performed for each experiment and the average value was considered for the analysis.

3. Results And Discussion

3.1. Identification of Completion of Fermentation Process

The measure of the specific gravity of the fermentation sample is one way of identifying the completion of the fermentation process. The initial specific gravity of the fermentable product will be higher and when fermentation starts, the value of specific gravity will keep on reducing with respect to the rate of fermentation. This drop-in specific gravity is a direct indicator of the fermentation of sugar into ethanol by yeast. 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 Fig. 4. Initially, the value of the specific gravity of the sample is 1.08 and at the end of the 4th day, its value reached 0.978 and remained stable. The total time taken for the completion of fermentation process was found as approximately 96 hours. This stabilized value of the specific gravity of the sample ensures the completion of the fermentation process.

3.2. Infrared Spectroscopy Results for Bioethanol

The IR spectroscopy result of the produced bioethanol sample is shown in Fig. 5. The graph depicts the presence of OH group (Alcohol functional group) in the produced sample. The peak at 3358.43 cm\(^{-1}\) is the evidence for the presence of the OH group. At the same time the broad OH vibration band in IR spectrum showed the existence of water molecules in the bioethanol sample. As a result of IR spectroscopy will not quantify the amount of ethanol produced, a gas chromatography test was carried out using the GC-MS system to ascertain the purity and quantity of ethanol production.

3.3. Gas Chromatograph Results for Bioethanol and Raw Oil

The gas chromatograph sample of the produced bioethanol is shown in Fig. 6. The peak value of the chromatograph sample and its comparison with the NIST library sample peak for ethanol are presented
in Fig. 7. From the chromatography analysis the % of bioethanol in the sample is calculated using the formula:

\[
\text{Percentage of Ethanol (\%) = \frac{\text{Individual peak area}}{\text{total peak area}} \times 100}
\]

From the calculation, it was found that 95.09% pure bioethanol was present in the sample with 4.9% water molecules. The molecular peaks of water molecules are observed between m/z 17.9 and 18.9. The yield of bioethanol from decayed *Manilkara zapota* fruits was found to be 10.45% (w/v).

The chemical constituents of Manilkara zapota seed oil were identified and quantified as palmitic acid 12.98%, stearic acid 5.12%, oleic acid 61.81%, linoleic acid 16.26%, linolenic acid 3.21%, and other unidentified components were 0.62%. The chromatograph result of the raw oil sample was presented in Fig. 8. Raw vegetable oil with high saturated fatty acid content will affect the cold flow characteristics of the biodiesel. The increased degree of saturation of the raw oil increases the viscosity of the biodiesel. Oil with increased poly unsaturated fatty acid will reduce the cetane value of the biodiesel. Hence, vegetable oils consist of low saturated & polyunsaturated fatty acids and high mono unsaturated fatty acids will be best suitable for biodiesel production (Sathish Kumar and Sureshkumar 2016). Manilkara zapota seed oil contains high mono unsaturated fatty acid (61.81% oleic) and low saturated and poly unsaturated fatty acids, hence best suitable for biodiesel production.

### 3.4. Optimization of Ethanolysis Process

#### 3.4.1. Effect of Bioethanol to Oil Molar Ratio

The effect of bioethanol to oil molar ratio on the yield of Manilkara zapota ethyl ester (MZEE) is shown in Fig. 9. The molar ratio in this analysis ranged from 3:1 to 15:1 in the 3:1 interval. The other two parameters catalyst quantity and process temperature were kept constant as 1% and 60°C respectively. It was observed clearly from Fig. 8 that the maximum MZEE yield was attained at a 9:1 molar ratio as 91.8%. The transesterification process is highly reversible, hence the stoichiometric molar ratio will not be good enough to result in higher biodiesel yield. Less reactivity of bioethanol in comparison with methanol was another reason for the higher amount of bioethanol consumption in this process. A noticeable reduction in biodiesel yield was recorded beyond the 9:1 molar ratio. This was due to the hydration caused by surplus alcohol present in the reaction which reduces the fatty acid ethyl ester conversion rate (Carmen et al. 2010; David et al. 2019; Hoang et al. 2009; Abdurrahman et al. 2008; Mahbub et al. 2011).

#### 3.4.2. Effect of Catalyst Quantity

The effect of catalyst quantity on the MZEE yield is presented in Fig. 10. Experiments were conducted by adjusting the quantity of the catalyst from 0.5 to 2.5% (w/w). The optimum value obtained from the previous segment was set at 9:1 for the bioethanol to oil molar ratio and other parameters were kept constant as before. The maximum *Manilkara zapota* ethyl ester yield was noted as 92.3% for 1.5% catalyst quantity. At 0.5% catalyst quantity, only 63.6% biodiesel yield was recorded. This was because of the insufficient quantity of catalyst to aggravate the mechanism of the reaction. Due to the higher degree of saponification formation of excess KOH and water molecules present in the bioethanol with
triglycerides, further, a rise in catalyst quantity adversely affects the MZEE yield, thus ethyl ester yield was drastically reduced and even higher soap formation was noted (Umer et al. 2008; Piyanuch and Sasiwimol 2010).

### 3.4.3. Effect of Process Temperature

One of the most significant transesterification process parameters is the temperature of the process. Figure 11 shows the variation of *Manilkara zapota* ethyl ester yield with respect to various process temperatures. Experiments were conducted on five distinct temperatures ranging from 50°C to 90°C with 10°C interval. The optimum bioethanol to oil molar ratio values and the quantity of catalysts obtained from previous segments have been introduced and other considerations have remained stable. From Fig. 10 it was found that the rise in temperature increases the MZEE yield up to 70°C. This was due to the higher temperature that allows coherent bonding between triglyceride molecules to be quickly braked and made active in transesterification conversion. (Sánchez et al. 2013; Schinas et al. 2009). A further rise in temperature had a reverse effect and reduced the biodiesel yield. This was due to the evaporation of bioethanol at 80°C and 90°C process temperature and would have not participated in the transesterification process. The optimal value of the MZEE yield was found to be 93.1% at 70°C.

### 3.5. Physicochemical Properties of Bioethanol, Raw Oil and *Manilkara Zapota* Ethyl Ester

Key properties of bioethanol such as 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 values and given in Table 1. The higher density of bioethanol as 873 kg/m$^3$ is noted due to the presence of small amount of water molecules in the bioethanol. The physicochemical properties of raw oil were estimated and presented as follows. The density of oil at 15°C was 0.895 g/cm$^3$, the kinematic viscosity at 40°C was measured as 33.97 mm$^2$/s, the acid value of the oil was estimated as 3.92 mg KOH/g and the free fatty acid value was calculated as 1.96% hence best suitable for single-step transesterification process. Free fatty acid content more than 2.5% in the oil leads to high soap formation with KOH during transesterification, hence two step transesterification process is needed. The iodine value of the oil was estimated as 64.3 g Iodine/100g. The physical appearance of the oil was clear brownish-yellow and the molecular weight was calculated as 873.31 g/mol. The physicochemical properties of *Manilkara zapota* ethyl ester were estimated and compared with *Manilkara zapota* methyl ester properties (Sathish Kumar et al. 2015) and given in Table 2. From the results, it was found that all the property values are comparable with methyl ester produced from *Manilkara zapota* oil and biodiesel standards.
Table 1
Physicochemical properties of Bioethanol in comparison with ethanol

| Parameters                  | Bioethanol | Ethanol  |
|-----------------------------|------------|----------|
| Density (kg/m$^3$)          | 873        | 789      |
| pH                          | 6.5        | 7.33     |
| Molecular weight (g/mol)    | 46         | 46.07    |
| Octane number               | 108        | 113      |
| Auto-ignition temp. (°C)    | 425        | 365      |
| Stoichiometric A/F ratio    | 9.00       | 9.00     |
| Flash point (°C)            | 23         | 16.63    |
| Heating value (MJ/kg)       | 26.9       | 27.3     |
| Freezing point (°C)         | -90        | -114     |
Table 2
Physicochemical Properties of Manilkara zapota ethyl ester (MZEE) in comparison with Manilkara zapota methyl ester (MZME)

| Properties                              | Test method | EN 14214 Limit value | MZEE  | MZME [23] |
|-----------------------------------------|-------------|-----------------------|-------|-----------|
| Ester content (% (m/m))                | EN14103     | Min 96.5              | 95.3  | 96.8      |
| Density at 15°C (g/cm³)                | ASTM D4052  | 0.86–0.90             | 0.889 | 0.875     |
| Kinematic viscosity (mm²/s)            | ASTM D445   | 3.5–5                 | 4.96  | 4.67      |
| Acid value (mg KOH/g)                  | ASTM D664   | Max 0.50              | 0.28  | 0.15      |
| Iodine value (g iodine/100 g)          | AOAC CD1-25 | Max 120               | 61.28 | 65.28     |
| Pour point (°C)                        | ASTM D97    | Max 0                 | -5    | -6        |
| Flash point (°C)                       | ASTM D93    | Min 120               | 178   | 174       |
| Heating value (MJ/kg)                  | ASTM D240   | Min 35                | 36.6  | 37.2      |
| Cetane number                          | ASTM D613   | Min 51                | 51    | 52        |
| Sulphur content (mg/kg)                | ASTM D5459  | Max 10                | 0     | 0         |
| Monoglyceride content (%(m/m))         | EN14105     | Max 0.8               | 0.71  | 0.52      |
| Diglyceride content (%(m/m))           | EN14105     | Max 0.2               | 0.16  | 0.13      |
| Triglyceride content (%(m/m))          | EN14105     | Max 0.2               | 0.14  | 0.12      |
| Free glycerol content (%(m/m))         | EN14105     | Max 0.02              | 0.00  | 0.00      |
| Total glycerol                         | EN14105     | Max 0.25              | 0.2   | 0.17      |

4. Conclusion

Decayed (waste) Manilkara zapota fruit pulp and skin collected from cultivation field and pulp, jam, and juice industries have been used for bioethanol production using Saccharomyces cerevisiae yeast through the fermentation process. The produced bioethanol was utilized to transform Manilkara zapota seed oil into Manilkara zapota ethyl ester (biodiesel). Three key parameters of the ethanolation process were optimized. The following conclusions were arrived based on the outcome of the research work:

- Decayed Manilkara zapota fruits can be a good substrate for bioethanol production.
- 200g of Manilkara zapota smash was found to produce approximately 21ml of bioethanol when 10gm of Saccharomyces cerevisiae (Baker’s Yeast), 50gm sucrose, and 1gm urea were added for the fermentation process. The maximum quantity of bioethanol obtained was found to be 10.45% (w/v).
- The GC-MS results infer that the purity of ethanol obtained in the sample is 95.09% by volume.
Bioethanol to oil molar ratio of 9:1, KOH quantity of 1.5% (w/w), and oil temperature of 70°C were identified as optimized ethanolsysis reaction factors. The maximum yield of *Manilkara zapota* ethyl ester was identified as 93.1%.

The physicochemical properties of *Manilkara zapota* based bioethanol and biodiesel show that they are a good substitute for fossil diesel and gasoline in overcoming the global alarming twin problems of environmental pollution and energy crisis.

This research work can be further extended by considering the following points in the future. The quality of the bioethanol from the decayed *Manilkara zapota* fruit can be improved by optimizing the fermentation process. The biodiesel quality can be further improved by implementing ultrasonic assisted transesterification process.

**Declarations**

**Ethics approval and consent to participate**

Not Applicable

**Consent for publication**

Not Applicable

**Availability of data and materials**

Not Applicable

**Competing interests**

"The authors declare that they have no competing interests"

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**Authors' contributions**

**R. Sathish Kumar:** Conceptualization, Methodology, Investigation, Resources, Validation, Writing - Original Draft

**K. Sureshkumar:** Conceptualization, Methodology, Writing - Review & Editing, Validation

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**Figures**
1. Reflux condenser
2. Clamp
3. Stand
4. Magnetic Stirrer with Hot plate
5. Conical Flask with Lid
6. Stirrer Pallet

Figure 1

Schematic Representation of Transesterification Experimental Setup

Figure 3

Fermentation Process in Incubator
Figure 6

Gas Chromatograph of Bioethanol Sample

Figure 7

Mass Spectrometry Result of Bioethanol Sample
Figure 9

Variation of MZEE Yield with Respect to Variation of Bioethanol to Oil Molar Ratio

Figure 11

Variation of MZEE Yield with Respect to Variation of Process Temperature