Extraction of Biodiesel from Microalgae

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ABSTRACT

Biodiesel is a renewable and clean fuel as it reduces carbon monoxide, carbon dioxide, hydrocarbons, and particulate matter emissions compared with petroleum-based diesel fuel. Biodiesel is a long-chain fatty acid ester made from renewed and biological raw materials such as used cooking, animal fat, vegetable oil, and algae. Production of biodiesel from renewable resources is done through the transesterification reaction at which the organic group (alkyl) of alcohol is substituted with the organic group of a triglyceride– the main component of the feedstock –producing fatty acid alkyl ester (biodiesel) and crude glycerol. Biodiesel can be used in pure form (B100) or may be blended with petroleum diesel at any concentration if its specifications is identical to the international standard specifications provided by American standard for testing materials (ASTM) or EN14214 in the European Union for alternative fuels. This study deals with different types of feedstocks treatment methods, and biodiesel production technologies. Various changes were made within the transesterification techniques that lead to varied physical properties of biodiesel and amounts of biodiesel.

Key words: Biodiesel, algae, waste cooking oil and transesterification.

1. INTRODUCTION

Energy occupies a very important place in the daily lives of humans globally. Domestic, industrial and transportation activities are completely dependent on energy availability[1,24]. Energy demand from these sectors is primarily met by fossil-based fuels whose utilisation has been reported to have several negative consequences that makes their continued use unsustainable[2]. This has motivated the search for environmentally friendly and sustainable alternatives and biofuels have been identified as one of such alternatives that can help meet global energy demand without the negative consequences associated with the use of fossil-based fuels[3]. Biodiesel has emerged and touted to have the capability of replacing petroleum-based diesel[4]. Biodiesel is commonly produced via transesterification of oils and fats which involves the reaction between the triglyceride present in the oil or fat with a suitable alcohol like methanol using a suitable catalyst to produce fatty acid alkyl esters (FAAE) referred to as biodiesel [5]. During this reaction, glycerol is usually a by-product which is separated from the produced biodiesel which itself is taken through further purification steps prior to storage and use[6-9]

Biodiesel and conventional hydrocarbon-based diesel blends are most commonly distributed for use in the retail diesel fuel marketplace [10-14]. The ASTM uses a system known as the "B" factor to state the amount of biodiesel in any fuel viz., 100% biodiesel is referred to as B100; 20% biodiesel, 80% petro-diesel is labeled B20; 5% biodiesel, 95% petro-diesel is labeled B5; 2% biodiesel, 98% petro-diesel is labeled B2[22,23].

1.1 Algae

Algae were said to be one of the best sources of biodiesel. These are the highest biodiesel yielding feedstock. It has the potential to produce 250 times the quantity of oil per acre as soybeans. Indeed, making biodiesel from algae could be the only option to supply enough automobile fuel to replace current gasoline consumption. Algae produce seven to thirty-one times more oil than palm oil[7]. Extracting oil from algae is a straightforward process. Microalgae is the best algae for biodiesel. Microalgae are photosynthesis-capable organisms with a diameter of less than 2 mm. Macroalgae, like seaweed, isn't extensively employed in biodiesel

manufacturing. Microalgae produces far more oil than macroalgae and grows considerably faster and more easily[9].

Microalgae can be used to produce a variety of sustainable biofuels. Methane is produced by anaerobic digestion of algal biomass, biodiesel is made from microalgal oil, and biohydrogen is produced photobiologically [16] . The idea of using microalgae as a source of fuel is not new, but it is now being examined seriously as a result of the rising price of petroleum and, more importantly, the growing worry about global warming caused by fossil fuel combustion [17].

1.2 Oil extraction processes

The extraction of oil is the most important step in biodiesel synthesis. In oil extraction, physical, chemical, or enzymatic treatments are applied to the plant to recover the oil. The major products include crude oil and cakes of the already-used plant. The cake produced in this step is not used in further processing. Different technologies viz., Mechanical extraction, Steam distillation, Solvent extraction, Soxhlet extraction, Chemical leaching, Enzymatic oil extraction, Supercritical fluid extraction, Microwave-assisted extraction, Ultrasound-assisted extraction, Transesterification reaction[18-21, 24].

1.3 Biodiesel purification:

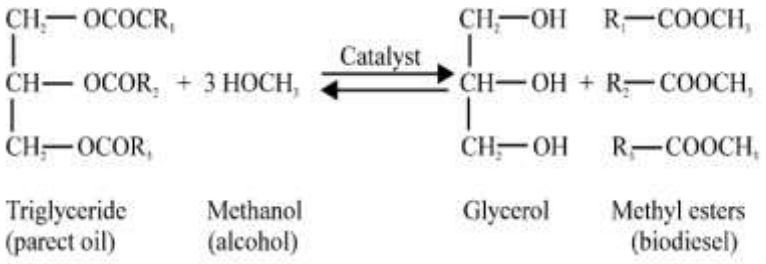
The first purification step of biodiesel is separation of biodiesel and glycerol by the difference in their densities as the biodiesel density is around 0.88 gm/cm^3 while density of glycerol is around 1.05 gm/ cm^3 [11,15]. The lower layer is glycerol and upper layer is biodiesel. The second step of biodiesel purification is the water washing of produced biodiesel with warm distilled water at ambient temperature. This aims in removing salts and soap formed during transesterification reaction[12-14,17]. The washing step is repeated until the biodiesel layer become clear and water layer become transparent[11].

2. Materials and methods:

2.1 Method A:

Collect two grams of algae from the freshly formed rain water pond in JNTUK surroundings and wash the sample thrice with 5% CH_3COOH. Ground the algae

with motor and pestle. Take small amount of algae from grounded algae in a tight container. Add 13 ml of Hexane and then mix with the dried ground algae and shake vigorously to extract oil. Dissolve 0.5 g NaOH in 24 ml methanol and stir properly for 20 min. Transfer the mixture of catalyst and methanol into the algal oil in a tight container for the transesterification process to takes place. The Triglycerides present in the algae react with methanol in the presence of NaOH catalyst would yeild the formation of biodiesel and glycerol. This reaction process is called transesterification. The mixture containing algal oil should be continously stirred.



After shaking, the solution is placed for 40 mins to settle the biodiesel and sediment layers clearly. Observe for the seperation of layers. The biodiesel should be separated from sedimentation carefully and wash biodiesel with 5% water. Repeat the washing until it become clean. Biodiesel production is stored for analysis.

2.2 Method B:

Collect Algae from small pond near JNTUK. The sample is pretreated with 5% CH_3COOH. Dry the algae for 20 min at 60°C in a incubator and grind to powder. Add Hexane and ether solution (20 and 20 mL) to the dried algae and mix vigorously to extract oil. Keep the mixture for settling for 24 hrs. To the separated oil, dissolve 0.25 g NaOH in 24 ml methanol and stir properly for 20 min.

Pour the mixture of catalyst and methanol into the algal oil in a tight container for Transesterification. The conical flask containing solution should be mixed properly for 3 h by electric shaker at 300rpm.

After the reaction, keep the solution for 15 mins to settle the biodiesel and sediment layers clearly. Observe for the seperation of layers. The biodiesel should be separated from sedimentation carefully and separate the sediment quantity

seperately (glycerine, pigments, etc.). Wash biodiesel with 5% water. Repeat the washing until it becomes transparent.

2.3 Method C:

Collect Algae from Pond near Bhavanarayana Swamy Temple, Kakinada. Pretreat the collected algae sample three times with 5% CH_3COOH. Dry and ground algae for 20 min at 60°C in a incubator to release water. 1g of algae powder extract is extracted using hexane as solvent. The powder is placed in a thimble. The Soxhlet extractor is used to extract a component from a mixture. The process requires a small amount of solvent to extract a higher amount of the targeted compound. It promotes solid and liquid to recover the desired compounds from the solid matrix using a suspension of a matrix into the refluxing solvent. This is repeated for 30 cycles.

The algae continuously receives the hexane solvent by the condensation of its vapors moving through the distillation arm. After solvent reaching a certain level in thimble, the algae treated hexane solution is taken from the cavity by a siphon moving back into the distillation chamber or boiling flask. The transesterification reaction is the main reaction that produces fatty acid alkyl ester (biodiesel); the byproduct is glycerol, which have a density higher than biodiesel density and hence settles at the bottom based on gravity. Chloroform to Methanol ratio of 1:2 is added to 10 ml soxhlet solution. Later, one gram of NaOH should be added slowly and carefully to the above mixture and agitated continuously to ensure a complete dissolving. After shaking, allow the solution for 15 mins to settle the biodiesel and sediment layers clearly. Collect and store the biodiesel

2.4 Method D:

The same sample of above method was pretreated, dried and soxhlet extracted as mentioned above for 30 cycles. This is underwent transesterification reaction that produces fatty acid alkyl ester (biodiesel) and the byproduct glycerol. Firstly, Chloroform to Methanol ratio of 1:2 is added to 10 ml soxhlet solution. and the mixture is taken in a separating funnel and allowed to settle for 60 mins. Then Chloroform layer should be separated and collected into an air tight container. Later, 1.5gms of NaOH and Methanol should be added slowly and carefully to the

above mixture and agitated continuously to ensure a complete mixing. Later, the solution is allowed to settle for 15 mins so that the biodiesel and sediment layers are separated clearly. The biodiesel should be separated carefully.

2.5 Phytochemical screening:

The phytochemical analysis was performed for the algae samples. The phytochemical screening was done to identify the presence of flavonoids, alkaloids, tannins, phenols, steroids, glycosides, proteins, carbohydrates and lipids. Dragendroff's test, Wagner's test for Alkaloids, Shinoda's test for flavonoids, Benedict's test, Molish test for carbohydrates, Lead-acetate, ferric chloride test for tannins, Salkowski test for terpenoids, Ninhydrin and test for proteins, foam test for saponnins, Emulsification test for lipids, Liebermann's test for glycosides. These phytochemicals are known to support bioactive activities in medicinal plants and are thus could be responsible for antioxidant activities of this plant.

2.5.1 Carbohydrates:

Benedict's test: 1ml of sample extract is treated with 1ml of Benedict's reagent and is allowed to heat for few minutes at temperatures 80-110^{0}C. Green color should be observed which means presence of reducing sugars.

2.5.2 Protiens:

Ninhydrin test: Take 1ml of sample in a test tube and add 1ml of ninhydrin reagent to the sample [pinch of Ninhydrin is dissolved in 10ml of ethanol]. The change in the color of solution turning to blue or violet will be observed, which indicates the presence of amino acids in the sample.

2.5.3 Alkaloids:

Dragendroff's test: 1ml of sample is treated with 1ml of Dragendroff's reagent and mixed well. The orange brown precipitate must be formed.

2.5.4 Wagner's test:

1ml of sample extract is treated with 1 ml of Wagner's reagent which would yield reddish brown precipitate.

The reddish brown precipitate will be observed which is considered as the presence of alkaloids.

2.5.5 Terpenoids:

Add 2ml of chloroform and 1ml of Sulphuric acid to 1mlof sample extract then allowed for heating in water bath for 2 minutes at 50^0C, grey color precipitate must be observed.

2.5.6 Tannins:

Lead acetate test: Pinch of lead acetate is dissolved in 1ml of distilled water (1% lead acetate) to which 1ml of plant extract is added to it, yellow precipitate should be observed.

2.5.7 Ferric chloride test:

2ml of Ferric chloride is added to 2ml of sample extract, which will show greenish black color.

2.5.8 Flavonoids:

NaOH test1ml of sample is added to 2% NaOH, yellow color will be observed.

2.5.9 Shinoda test:

2ml of sample is added to 5ml of ethanol and 2ml of HCl, Mg Turnings. Slight pink color will be formed.

2.5.10 Saponins:

Foam test: 0.5ml of sample is added to 4.5ml distilled water and is shaken vigorously. Honey comb froth will be observed.

2.5.11 Steroids:

Salkawski's test: 1ml of sample is taken and is treated with 2ml of chloroform and 1ml of sulphuric acid. Upper layer is reddish shade and bottom is yellow with greenish particles type will be observed.

2.5.12 Glycosides:

Add 1 ml of extract with 1ml of chloroform and 1ml of acetic acid and ice cool it for 2 minutes. Now add 5-7 drops of sulphuric acid and observe for violet to blue to green color change.

2.5.13 Lipids:

Emulsification test: Take 5ml of sample extract and add 5ml of chloroform and 10ml of methanol (2:1 ratio), place the solution in a separating funnel. Separate the upper and lower layer using funnel extraction method. Collect the bottom layer.

Take equal volumes of ethanol and water into 2 different eppendorf tubes. Add equal amount of sample (upper funnel extract) into 1 eppendrof. Also add equal amount of sample (lower funnel extract) into another eppendorf. The result will be observed as cloudy appearance after mixing.

2.6 Biodiesel properties testing :

2.6.1 Free fatty acid and acid number

The free fatty acid (FFA) content and acid number were determined according to AOCS method. Oil sample (1 g) was weighed into a flask followed by addition of 95% ethanol and 2 ml phenolphthalein indicator. The sample containing ethanol should be neutralized by adding NaOH until a faint pink color appeared. The sample should be continued titrating against sodium hydroxide until the appearance of a permanent pink color of the same intensity as that of neutralized ethanol (before the addition of the sample). As a precautionary measure, the permanent pink color was allowed to persist for at least 30 s during titration. The free fatty acid content and acid number was calculated as:

$$\text{Free fatty acid (\%)} = (\text{ml of alkali} \times N \times 28.2)/w$$

where N is normality of NaOH solution, w is the weight of oil (g).

$$\text{Acid number (mg KOH/g)} = 1.99 \times \text{FFA(\%)}.$$

3. Results and discussions:

3.1 Method A:

Figure 1: Collection of algae sample 1

Two grams of algae were collected from the freshly formed rain water pond in JNTUK surroundings (Figure 1). Collected sample were washed and grounded with motor and pestle. Hexane(13ml) was then added to algae sample and shaked vigorously. 0.5 g of NaOH was dissolved in 24 ml methanol and stirred. Mixture of catalyst and methanol were then poured into the algal oil in a tight container and stirred continuously for the Transesterification reaction to take place. The solution was kept undisturbed and the layers separation was observed. Finally Biodiesel production were measured and stored for analysis (figure 2).

Figure 2: Biodiesel production from algae method 1

In the obtained biodiesel, Red and blue flame was observed with high intensity and less propensity to spread. Flame lasted for short duration of 6 s. Yield of 18.42% was reported from this method.

3.2 Method B:

Figure 3: Collection of algae sample 2

Algae was collected from Stagnant small pond formed near JNTUK surroundings (figure 3). Collected sample were washed and grounded with motor and pestle and then dried in an incubator. Equal amounts of hexane and ether solutions were added to the dried algae, mixed vigorously and kept for settling. Settled Biomass was then filtered. 0.5 g of NaOH was dissolved in 24 ml methanol and stirred. Mixture of catalyst and methanol were then poured into the algal oil in a tight container and stirred continuously in electric shaker at 300rpm for the Transesterification reaction to takes place.

The solution was kept undisturbed and the layers separation was observed. The Biodiesel was separated from sediment layers carefully and washed with 5% water. Finally, Biodiesel production were measured and stored for analysis (figure 4).

Figure 4: Biodiesel production from algae method 2

In the obtained biodiesel, less gas with low temperature and pressure was observed in the tight container. Red and blue flame was observed with high intensity and

medium propensity to spread. Flame lasted for short duration of 7 s. Yield of 4.68 % was reported from this method.

3.3 Method C

Algae sample was collected from Pond near Bhavanarayana Swamy Temple, Kakinada. Collected sample were washed and grounded with motor and pestle and then dried in an incubator. Algal oil was extracted using Soxhlet extraction process where, dried algae is placed in the filter paper and Hexane was used as a solvent and the extraction process is repeated for 30 cycles. Chloroform to Methanol ratio of 1:2 was added to 10 ml soxhlet solution. One gram of NaOH was added to the above mixture and agitated continuously for the Transterification reaction to takes place.

The solution was kept settle and the layers separation was observed. The Biodiesel was separated from sediment layers carefully and washed with 5% water (figure 5). Finally, Biodiesel production were measured and stored for analysis.

Figure 5: Biodiesel production from algae method 3

In the obtained biodiesel, very high temperature and more gas with pressure was observed while releasing the tight container. High intensity and longlasting blue flame with moderate propensity to spread was observed. Flame lasted for 12 s. Yield of 37.5 % was reported from this method.

3.4 METHOD D

Algae sample was collected from Pond near Bhavanarayana Swamy Temple, Kakinada. Collected sample were washed and grounded with motor and pestle and

then dried in an incubator.Algal oil was extracted using Soxhlet extraction process where, dried algae is placed in the filter paper and Hexane was used as a solvent and the extraction process is repeated for 30 cycles. Firstly, Chloroform to Methanol ratio of 1:2 was added to 10 ml soxhlet solution and the mixture was taken in a separating funnel and kept settled. Then Chloroform layer was separated and collected into an air tight container. Secondly, 1.5gms of NaOH and Methanol was added to the above mixture and agitated continuously for the Transterification reaction to takes place.

The solution was kept undisturbed and the layers separation was observed. The Biodiesel was separated from sediment layers carefully and washed with 5% water (figure 6). Finally, Biodiesel production were measured and stored for analysis.

Figure 6: Biodiesel production from algae method 4

In the obtained biodiesel, very high temperature with more release of pressured gas was observed while releasing the tight container. High intensity and longlasting blue flame with moderate propensity to spread was observed . Flame lasted for 12 s. Yield of 55 % was reported from this method.

3.2 Phytochemical screening

Dry algae sample was placed in the thimble or filter paper and is underwent soxhlet extraction. To this, Hexane is used as a solvent. The algae continuously receives the hexane solvent by the condensation of its vapors moving through the distillation arm. After solvent reaching a certain level in thimble, the algae treated hexane solution is taken from the cavity by a siphon moving back into the

distillation chamber or boiling flask. This extraction process is repeated for 30 cycles and extract was used for phytochemical screening (table 1). The results showed that the sample contains only primary metabolites i.e Carbohydrates, Protiens and lipids.

Table 1: Phytochemical analysis for Secondary metabolites

Metabolites	**Test Result**
Carbohydrates	Positive
Proteins	Positive
Lipids	Positive
Alkaloids(Dragondroff's test)	Negative
Alkaloids(Wagner's test)	Negative
Terpenoids	Negative
Tannins(Lead acetate)	Negative
Tannins(Ferric Chloride test)	Negative
Flavonoids(NaOH test)	Negative
Flavanoids(Shinoda test)	Negative
Saponnins(Foam test)	Negative
Steroids	Negative

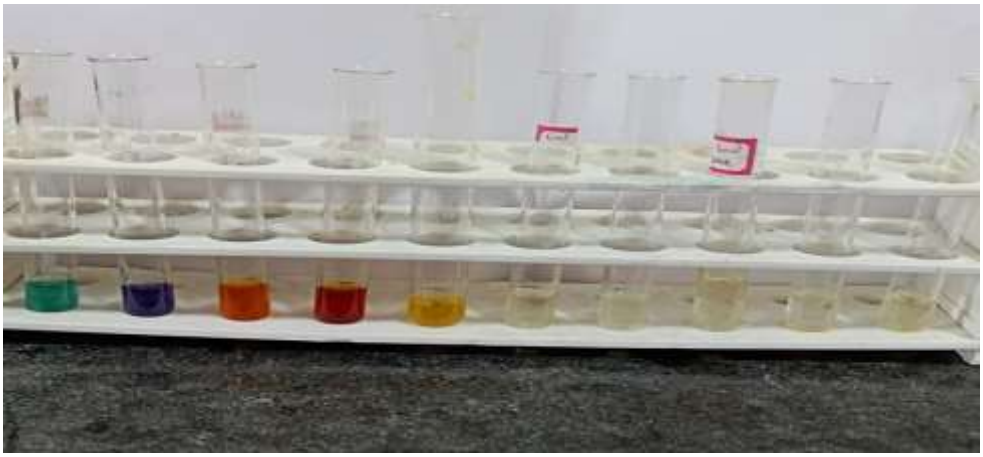


Figure 7: Qualitative tests for Secondary Metabolites.

3.3 Biodiesel properties testing:

Figure 8: Titration of Biodiesel oil sample for Acid and FFA value

3.3.1 Free fatty acid and acid number

Oil sample (1 g) was weighed into a flask followed by addition of 95% ethanol and 2 ml phenolphthalein indicator. The sample was continuously titrated against sodium hydroxide until the appearance of a permanent pink. The free fatty acid content and acid number was calculated as:

$$\text{Free fatty acid (\%)} = (\text{ml of alkali} \times N \times 28.2)/w$$

where N is normality of NaOH solution, w is the weight of oil (g).

Acid number (mg KOH/g) = 1.99 ×FFA(%).

Acid and fatty acid tests for algal biodiesel:

The optimal Acid value is expressed as mg KOH required to neutralise 1g of biodiesel. The maximum acid value should be no more than 0.50 mg KOH/g according to ASTM standards [80]. According to this, biodiesel produced by method 3 and 4 in algae satisfied the ASTM standards (Table 2) which is graphically represented in figure 9.

Table 2: Acid and Fatty acid Tests for different methods of Algal Biodiesel

Methods	Acid number value(mg KOH/g)	Fatty acid values (%)
Method 1	5.6118	2.82
Method 2	6.172	3.102
Method 3	0.05	0.0282
Method 4	0.049	0.025

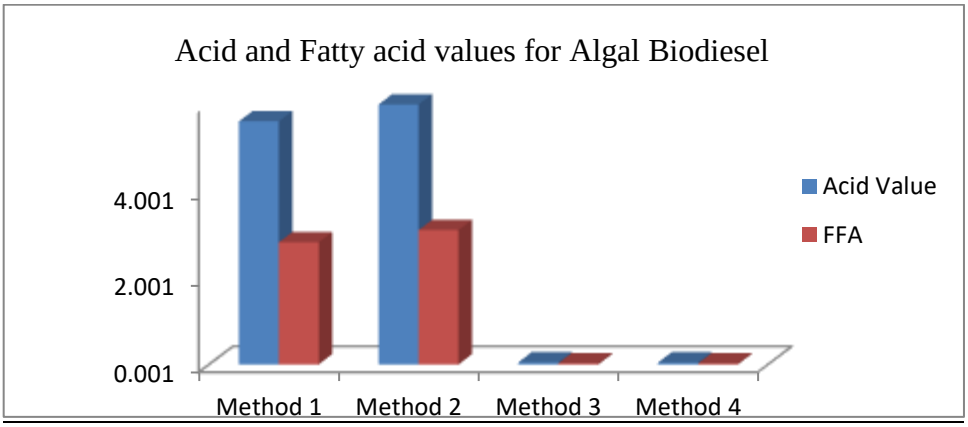


Figure 9: Graph plotted for Acid and Fatty acid Tests for Algal Biodiesel.

4. Conclusion

Biodiesel is a promising and more attractive fuel for diesel engines owing to its renewable nature and environmental benefits. The key issue to take into consideration is the higher price of biofuels than fossil fuel. Using low-quality feedstocks –which do not compete with food supply and land for food cultivation

such as non-edible oils is considered an effective way of reducing the biodiesel production costs.

In the present research, the algae feedstock from bhavanarayana temple has been observed to produce 37.5% biodiesel with 1:2 of Chloroform to Methanol added to soxhlet extract. Very high temperature and gas release was observed during the transesterification process. High intensity and longlasting blue flame with moderate propensity to spread was observed with this biodiesel.and the flame lasted for 12s.

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