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Research Paper

PRODUCTION AND OPTIMIZATION OF BIODIESEL FROM DIFFERENT GENERATIONS

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Abstract

Biodiesel is triglycerides derivative or free fatty acids which produced by transesterification with short chain of alcohols. Biodiesel can be produced from different raw materials like waste engine oil, and waste cooking oil. In present study biodiesel was produced by two methods, viz. biological and chemical. Biological method includes the use of lipase as a catalyst where as chemical method utilizes conc. H_2SO_4 as a catalyst. The lipase producing fungi was isolated from oil spilled soil. Lipase was optimized, partially purified using ammonium sulphate precipitation method. Proficient lipase producing fungi was identified as Penicillium sp. on the basis of pigment, morphological characteristics and showed lipase activity 91.80±1.2 Uml^{-1}. The present considered lipase has the application in Biodiesel production. In both method, pre warmed substrate was mixed with 45% methanol in 6:1 ratio this mixture was incubated at 50°C for 2 hours. This yields the biodiesel and the unreacted substrate was used for the second cycle. In the separating funnel 3 layers were obtained viz. unreacted substrate, biodiesel and glycerine. Biodiesel was purified using column chromatography and characterized by various tests like qualitative and quantitative tests. The best result was obtained from fourth generation and the cost value was Rs. 50.

Key words: biodiesel, lipase, triglycerides, transesterification.

INTRODUCTION

The major part of all energy consumed in most parts of the world comes from fossil sources such as petroleum, coal and natural gas. However, these non-renewable sources will be exhausted in near future. Thus, the search for alternative sources of renewable and sustainable energy has gained significance with the potential to solve many current social issues such as the rising price of petroleum crude and environmental concerns like air pollution and global warming caused by combustion of fossil fuels [40].

Biodiesel, with the properties of low pollution, high fuel value which is safer than fossil diesel, is becoming the most promising alternative energy source for crude oil. Microorganism with short life cycle has drawn peoples' attention because it is easy to raise, suitable for high density fermentation, and easy to be mutated. Using oil producing microorganism to transform substrate to oil has become the best solution for sustainable development of biodiesel oil [49].

The most common way to produce biodiesel is to transesterify triglycerols in vegetable oil or animal fats with an alcohol in presence of an alkali or acid catalyst. Methanol is the commonly used alcohol in this process, due in part to its cost. The products, fatty acid methyl esters (FAME), are called biodiesel and include glycerine as a by-product (Zhang et al., 2003).Biodiesel is non-toxic and biodegradable substitute fuel that is obtained from non-renewable sources [31].

Choosing a proper feedstock is therefore crucial. Lipids obtained from non-edible feedstock are popular because they do not compete with the food market. Also, the prohibitive cost of edible oils prevents their use in biodiesel preparation. This is while non edible oils are affordable for biodiesel production. Non edible oils which have been used for biodiesel include Jatropha and Pongamia. Microalgae beings a non edible lipid source, is another potential candidate for biodiesel production as it does not compete with food commodities and has high lipid content which is usually between 20-50%. Studied have focused mostly on eukaryotic species such as *Botryococcus braunii*, *chlorella sp*., *Chlamydomonas reinhardtii* and *Nannocloropsis sp*. because of their relatively higher lipid content. Cyanobacteria- a microalgae prokaryotes is also gaining momentum in the biodiesel production arena with respect to its fast growth rate and lipid content [33].

Microalgae are sunlight driven cell factories that convert carbon dioxide to potential biofuel, foods, feeds and high value bioactivities. In addition, these photosynthetic micro-organisms are useful in bioremediation application and as nitrogen fixing biofertilizer. Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass. Biodiesel derives form microalgal oil and photobiologically produced biohydration. The idea of using microalgae as a source of fuel is not new but it is now being taken seriously because of the escalating price of petroleum and more significantly, the emerging concerns about global warming that is associated with burning fossil fuels [74].

Biofuel particularly biodiesel is such a fuel that shows great potential to replace petro-diesel. Biofuel are commonly known as offer several advantages over fossil fuel. Replacing petro-diesel with biodiesel fuel could reduce the accumulation of green houses such as CO_2 in the atmosphere. Also biodiesel fuel has been commonly found to offer similar engine emissions of particulates, hydrocarbon and carbon monoxide [27].

An alternative path for the production of biodiesel involves the use of enzymes as catalysts. The enzymatic catalyzed reaction generates fewer waste streams, and enzymes can convert both triglycerides and free fatty acids into biodiesel, resulting in an efficient process, under mild reaction conditions. Phase stability leads to a product with few impurities, and the aqueous phase which contains the catalyst is very stable. Furthermore, the reaction does not require solvents, and hence there is no need for a special treatment to recover the solvent for reuse. The enzymatic catalyzed process requires less energy but it has some drawbacks, such as the high cost of enzymes and the need for a careful control of reaction parameters [37, 17 and 3].

MATERIALS AND METHODS

Collection of sample

Soil samples were collected from oil spilled and petrol pump (Narmada auto Services, Satara Parisar, Aurangabad) and raw materials like waste engine oil from Maharashtra garage, N4-CIDCO Aurangabad, waste cooking oil from Appa tea stall, N3-CIDCO, Aurangabad, Cotton seed. Chemicals Methanol, conc. H_2SO_4, activated charcoal and all other reagents used were of analytical grade.

Biodiesel production by biological and chemical method

The raw material (waste engine oil, cotton seed oil and waste cooking oil) were pre-warmed at 50ºC for 20mins then 45% methanol is added in the pre warmed raw material in the ratio of 3:1, 6:1 then crude lipase (20ml), 0.5% H_2SO_4 (7.5ml) as a catalyst in biological and chemical method respectively, then kept for transesterification reaction at 50ºC for 2 hr in shaking condition, followed by separation in separating funnel for 6 hr. After separation three layers are observed viz., unreacted substrate, biodiesel and glycerine (Bueso et al., 2015). Obtained Biodiesel further proceed for optimization study.

Optimization parameters for Biodiesel production

All optimization parameters were carried out in triplicates.

[1] Effect of methanol and substrate on Biodiesel production:

Effect of methanol on biodiesel production was studied by the addition of different concentration of methanol, methanol: substrate and substrate: 45% methanol like 35%, 40%, 45%, 55%, 2:1, 4:1, 6:1, 8:1 and 1ml, 2ml, 4ml, 8ml respectively and proceed for transesterification reaction, further progress for analysis[40].

[2] Effect of catalyst (H_2SO_4) on Biodiesel production:

Effect of catalyst was evaluated by adding H_2SO_4 in different percentages like 0.25%, 0.5%, 1%, 1.5% and the catalyst effects is also checked by adding 0.5% H_2SO_4 in different volume like 0.15ml, 0.3ml, 0.6ml, 0.9ml respectively and proceed for transesterification reaction, further carry on for analysis [40].

Biodiesel confirmatory tests

[1] Fourier Transform Infrared Spectroscopy (FTIR):

FTIR of produced biodiesel, waste engine oil, std. biodiesel and petroleum diesel was analyzed at Department of Chemistry in Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.[33]

[2] 3/27 Biodiesel conversion test:

3ml of biodiesel was added to 27ml of methanol, stirred for about 30 seconds and allowed to settle for 5mins. The methanol should completely absorb the biodiesel, no fall out should be visible [26]

[3] Qualitative test for water content (Hot Pan test):

Small amount of biodiesel was added to heated pan. If bubbles, crackles and steam produce, it likely contains water molecules [26].

[4] Quantitative test for water content:

50ml biodiesel was weighed, and then heated upto 121°C with continuous stirring. After reaching the temperature, cool it for 10mins and reweigh the sample to calculate the water content [26].

[5] Biodiesel titration (Acid content):

1ml of biodiesel was mixed with 10ml of isopropanol add 2-3 drops of phenol red as pH indicator and titrate with 0.1N NaOH upto the solution colour does not change [26].

[6] Calorific value of Biodiesel:

Take weight of empty small petriplate add 1ml of biodiesel in the plate and reweigh it an place it in another petriplate containing 10ml of D/W, take a weight of the plates containing sample+ D/W. checked initial temperature of D/W, again checked temperature of D/W after burning of biodiesel and calculate the calorific value [25].

[7] Flash and Fire point of Biodiesel:

50ml biodiesel was taken in a beaker, checked its initial temperature, heat was supplied from surrounding slowly until the biodiesel catches fire itself [22].

[8] Pour and Cloud point of Biodiesel:

Small amount biodiesel was taken in test tube or screw cap tube, checked its initial temperature, incubate at 4°C and note down its temperature after every 15mins upto the jelly state is obtained [22].

[9] Specific gravity of Biodiesel:

Weight of empty specific gravity bottle was taken, then weight of specific gravity bottle with D/W. After that the specific gravity bottle with biodiesel was weighed and the specific gravity of biodiesel was calculated.

[10] Flame Test:

1ml of biodiesel was burnt and the flame was observed. (Anonymous)

RESULTS

3.1 Biodiesel production by biological and chemical method:

Biodiesel was successfully produced from waste engine oil, cotton seeds oil and waste cooking oil through transesterification process by using both chemical and biological method (Figures 1a and 1b). Crude biodiesel was purified by column chromatography using activated charcoal. The effluent was collected and used as biodiesel.

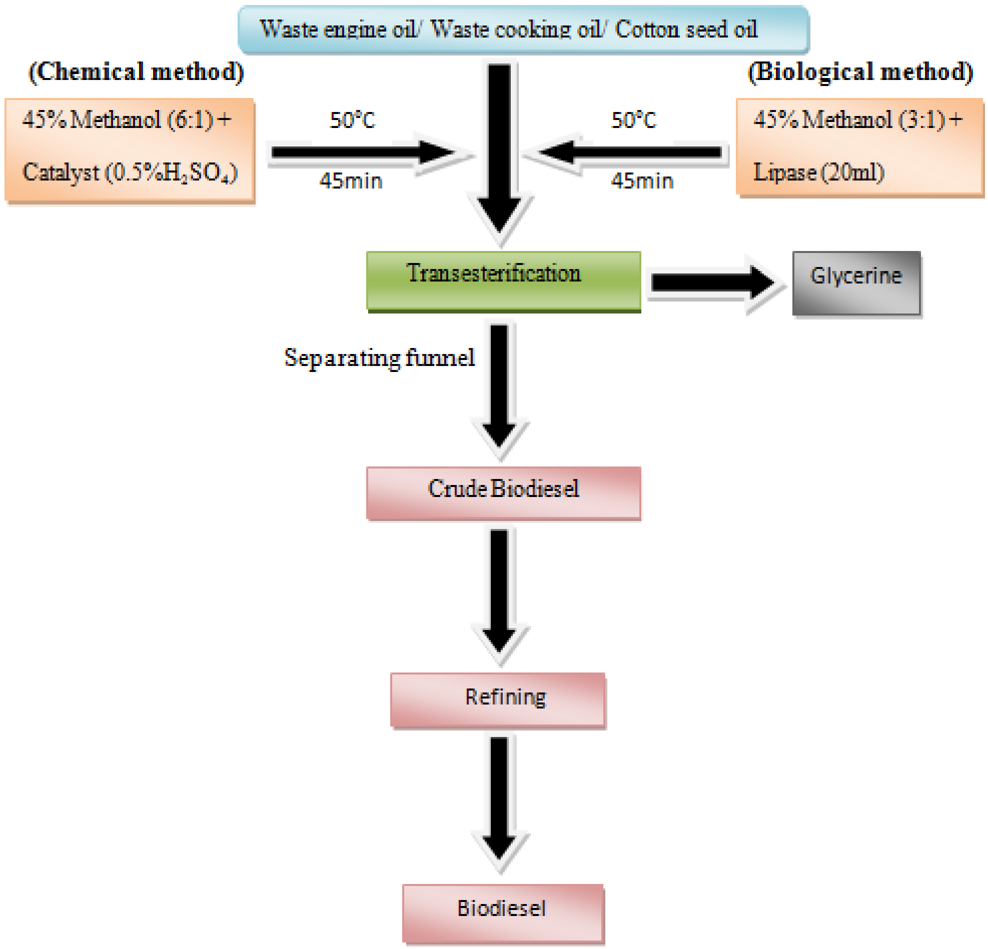

Figure 1a Schematic presentation of Biodiesel production

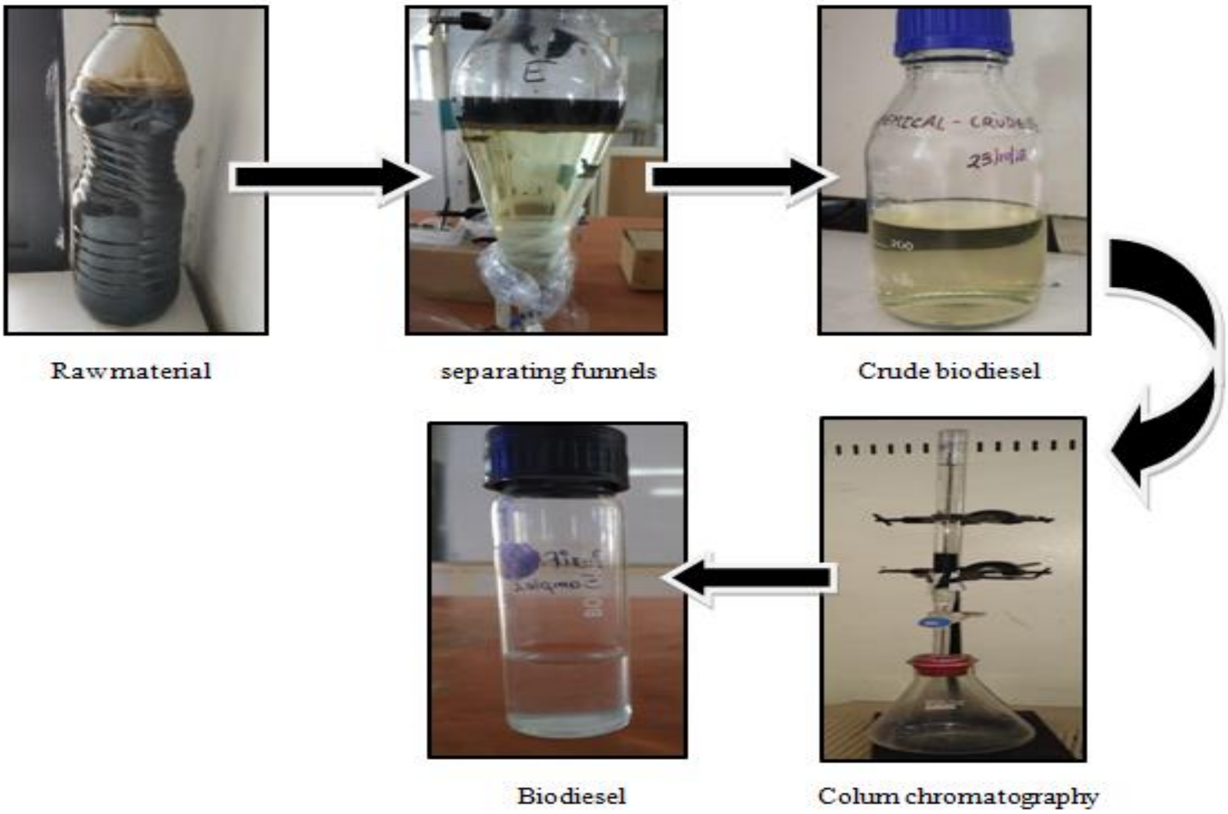

Figure 1b Schematic presentation of Biodiesel production

3.2 Optimization parameters For Biodiesel Production:

3.2.1 Effect of methanol and substrate on Biodiesel production:

In the study, as we increases the concentration of methanol, methanol: substrate and substrate: 45% methanol ratio it does not affect the biodiesel production. The 55% methanol and 8:1 ratio have strong smell.

3.2.2 Effect of catalyst (H_2SO_4) on Biodiesel production:

In the investigation of effect of catalyst on biodiesel does not affect the production by increasing or decreasing the different percentage and volume of H_2SO_4.

3.3 Biodiesel confirmatory tests:

3.3.1 Fourier Transform Infrared Spectroscopy (FTIR):

From the FTIR analysis of biodiesel and waste engine oil, their transmittance shows the different groups in biodiesel and waste engine oil it means the transesterification reaction are successfully occur. In biodiesel, the alcohol, phenol, fluoride, aldehyde, alkynes, ethers, esters, carboxylic acid, anhydrides groups are found at 3298 cm^{-1}, 1013 cm^{-1}, 2951 cm^{-1}, 1112cm^{-1}(Figures 2-6).

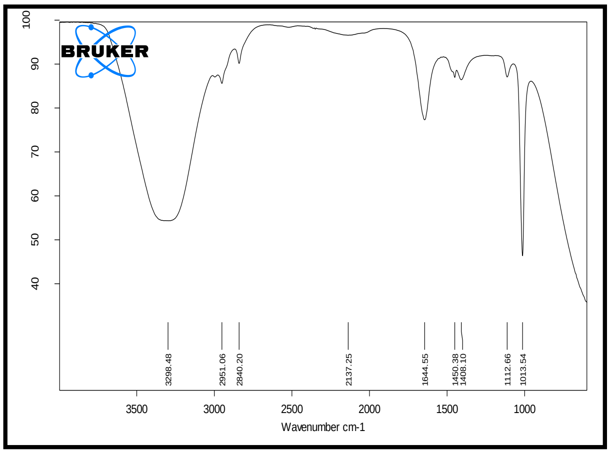

Figure 2 FTIR of Biodiesel

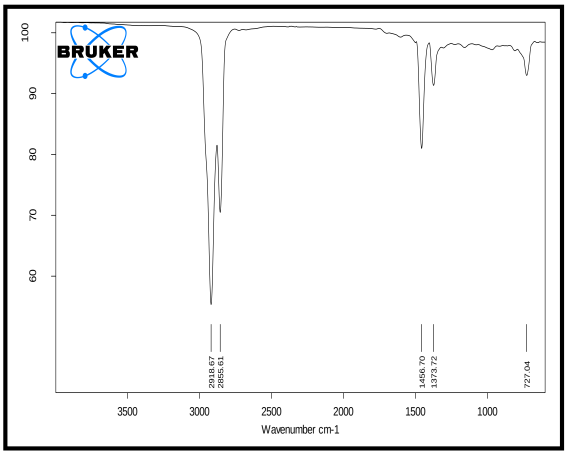

Figure 3 FTIR of Waste engine oil

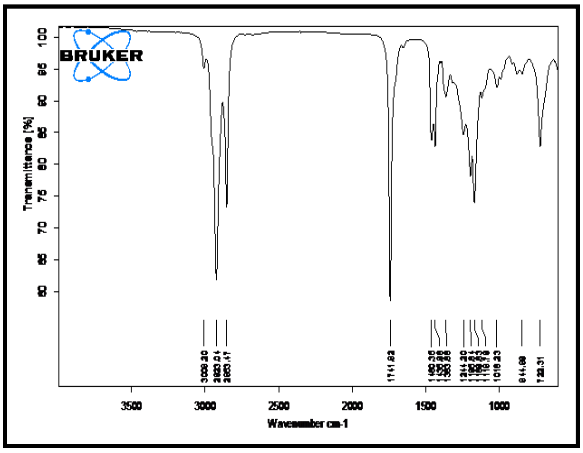

Figure 4 FTIR of Standard Biodiesel 1

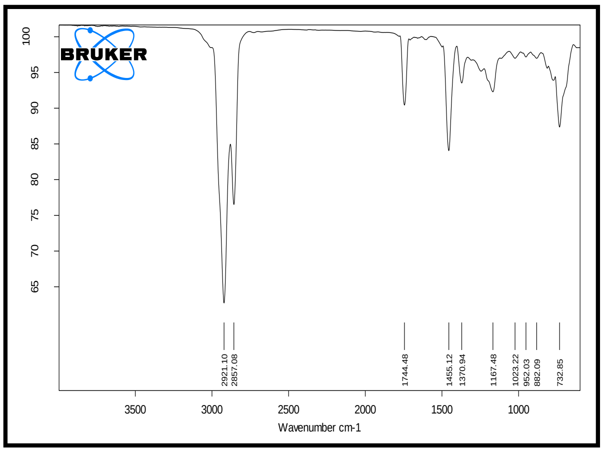

Figure 4 FTIR of Standard Biodiesel 2

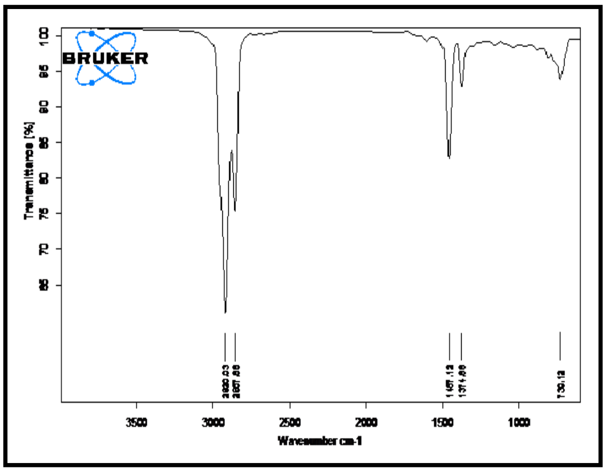

Figure 6 FTIR of Standard Diesel

3.3.2 3/27 Biodiesel conversion test:

After the 5mins the biodiesel observe clear transparent tube and compare with the standard biodiesel, the unreacted oil visible at bottom of the tube (Figure 7).

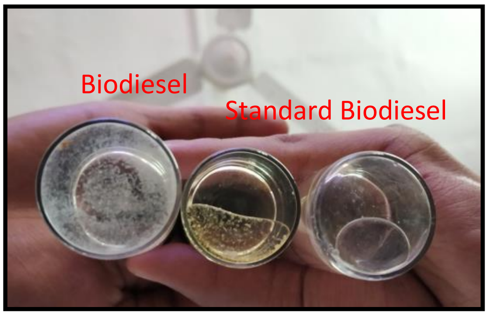

Figure 7 3/27 conversion tests

3.3.3 Qualitative test for water content (Hot Pan test):

The small amount of biodiesel was poured in hot pan, the bubbles, crackles and steam were observed indicating presence of water in biodiesel.

3.3.4 Quantitative test for water content:

After the calculations the water content in biodiesel was found to be 14000 ppm.

Calculation of water content:

Weight of empty beaker: 98.38gm

Weight of beaker+ biodiesel (before heat): 142.08gm

Weight of beaker+ biodiesel (after heat): 140.08gm

Water content= weight after heat- weight before heat = 2gm

Percent of water = water content/ weight before heat x 100

=2/ 142.08 x100

=1.4

Parts per million (ppm) = 1.4×10000

= 14000

3.3.5 Biodiesel titration (Acid content):

The acid content of biodiesel was found to be 1.683ml gm^{-1}.

Calculation of Acid content:

Acid value= 56.1 V × N / W

Where, V= volume in ml of NaOH used

N= Normality of NaOH

W= Weight in gm of sample.

Acid value= 56.1 (0.3)×0.1/1

=16.83×0.1/1

=1.683 ml gm^{-1}

3.3.6 Calorific value of Biodiesel:

The calorific value of biodiesel was found to be 1.59 MJ kg^{-1} of waste engine oil, 1.37 MJ kg^{-1} of waste cooking oil, 1.23 MJ kg^{-1} of cotton seed oil.

3.3.7 Flash and Fire point of biodiesel:

The flash point of biodiesel was found to be 50°C and fire point was 52°C.

3.3.8 Pour and Cloud point of biodiesel:

The biodiesel did not form jelly state within 24hr of incubation.

3.3.9 Specific gravity of biodiesel:

The specific gravity of biodiesel was found to be 0.953 gm cm^{-3}.

Calculation for specific gravity:

Weight of empty specific gravity bottle: 19.52gm

Weight of bottle+ D/W: 29.18gm

Weight of bottle+ biodiesel: 28.73gm

Specific gravity= weight of biodiesel/ weight of D/W

= 9.21/9.66

= 0.953 gm cm^{-3}

3.3.10 Flame Test:

Blue colour flame was observed for 1 min of biodiesel produced from waste engine oil (Figure 8).

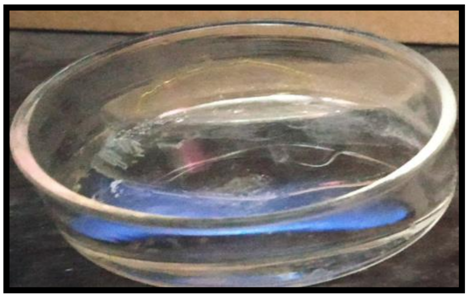

Figure 8 Flame Test

DISCUSSION

Biodiesel was successfully produced from waste engine oil, cotton seeds and waste cooking oil through transesterification process by using *Penicillium* fungal lipase as a catalyst. Crude biodiesel was purified by column chromatography using activated charcoal. The effluent was collected and used as biodiesel. From results of study by Bueso et al. [16], biodiesel was produced from jatropha by using lipase as a catalyst.

In optimization study of biodiesel production, the 8:1 ratio of methanol: waste engine oil gives the best result. Koh et al. [40], reported that 5:1 and 6:1 ratio of methanol: jatropha oil gives the best result in biodiesel production. The 55% methanol concentration has strong smell as compared to 45%, 35% & 40% methanol. 1.5% H_2SO_4 as a catalyst gives 90-95% conversion of waste engine oil to methyl ester in contrast with 0.25%, 0.5% & 1% H_2SO_4. Koh et al., [40], in their study stated that 1.0-1.4% NaOH and 0.55-2.0% KOH as a catalyst gives 90-99% of jatropha oil to methyl ester i.e. biodiesel.

The characterization of biodiesel was done by the FTIR, we found the alcohol, phenol, fluoride, aldehyde, alkynes, ethers, esters, carboxylic acid, anhydrides groups are found at 3298 cm^{-1}, 1013 cm^{-1}, 2951 cm^{-1}, 1112cm^{-1}. Beetul et al., [33], in their research stated that the peak at 1249.31 cm^{-1}, 1738.30 cm^{-1} and 1737.84 cm^{-1}, 1189.43 cm^{-1} indicate the presence of fatty acid methyl esters in the biodiesel mixture from the *Leptolyngbya sp.* and *Nodularia harveyana*.

Biodiesel production was confirmed by 3/27 biodiesel conversion test shows no fall out after the 5min, in the standard biodiesel the unreacted oil was found at the bottom of the tube. The bubbles, crackling and vapour steam was observe in qualitative test for water content (Hot pan test) after that quantitative water content is calculated and it found to be 14000 ppm. The acid content of biodiesel was found to be 1.683 ml gm^{-1}. The calorific value of waste engine oil is 1.59MJ kg^{-1}, waste cooking oil is 1.37MJ kg^{-1}, and cotton seed oil is 1.23MJ kg^{-1}. The flash point of biodiesel 50°C and fire point was 52°C. The pour point was not obtain due to biodiesel was not form jelly in the overnight of incubation at 4°C. The specific gravity of biodiesel is 0.953 gm cm^{-3}. Hanny and Shizuko [22] found flash point of biodiesel prepared from jatropha to be 37°C with respect to ASTM.

In present study the cost price of obtained biodiesel was rupees 50 per litre. The catalytic activity of bimetallic Gold–silver core–shell nanoparticles toward biodiesel production from Sunflower oil through transesterification was determined and is considerably costly method mentioned by Banerjee et al., [45].

CONCLUSION

The biodiesel was successfully produced by using a fungal lipase. The effect of methanol and H_2SO_4 as a catalyst was found to be enhanced at 55% methanol, 8:1 methanol: substrate ratio, 1.5% H_2SO_4 and 0.9ml of 0.5%H_2SO_4.From the FTIR, the alcohol, phenol, fluoride, aldehyde, alkynes, ethers, esters, carboxylic acid, anhydrides groups are found at 3298 cm^{-1}, 1013 cm^{-1}, 2951 cm^{-1}, 1112cm^{-1}.The 3/27 biodiesel conversion test shows the no fall out after the sitting of 5mins. The 14000 ppm water content present and acid content was 1.683 ml gm^{-1} in biodiesel. The calorific value was found to be 1.59MJ kg^{-1} of waste engine oil, 1.37MJ kg^{-1} of waste cooking oil, 1.23MJ kg^{-1} of cotton seed oil. Flash point and fire point was 50°C, 52°C respectively. Pour and cloud point was not obtain as the biodiesel did not form jelly state after overnight of incubation. Specific gravity of biodiesel was 0.953. The cost price of produced biodiesel was found to be 50 Rs.

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