

Production of Biodiesel using waste temple oil from Shani Shingnapur temple (Dist. Ahmednagar), Maharashtra, India using chemical and biological methods

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Abstract—In India, due to various mythological and religious reasons hundreds of devotees pour oil over the idols in Hanuman or Maruti and Shani temples. The oil once poured cannot be reutilized and was ultimately wasted. These waste temple oil from Shani Shingnapur was used to produce biodiesel. Immobilized *Pseudomonas aeruginosa* was used to catalyze transesterification of waste temple oil. The cells of *P.aeruginosa* were immobilized within the sodium alginate. Biodiesel production and its applications were gaining popularity in recent years due to decreased petroleum based reserves. Biodiesel cost formed from waste temple oil was higher than that of fossil fuel, because of high raw material cost. To decrease the cost of biofuel, waste temple oil was used as alternative as feedstock. It has lower emission of pollutants; it is biodegradable and enhances engine lubricity. Waste temple oil contains triglycerides that were used for biodiesel production by chemical and biological method. Transesterification reaction of oil produces methyl esters that are substitutes for fatty acid alkyl biodiesel fuel. Characteristics of oil were studied such as specific gravity, viscosity, acid number, saponification number. Parameters such as temperature, oil: methanol ratio were studied and 88%, 96% of biodiesel yield was obtained with effect of temperature and oil: methanol ratio on transesterification reaction. With addition of NaOH or KOH to fatty acids which formed salt known as soap, which is excellent emulsifying and cleaning agents.

Keywords— Biodiesel, catalyst, immobilization, transesterification.

I. INTRODUCTION

Biodiesel is defined as a fuel composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats [24]. A mono-alkyl ester is the product of the reaction of a straight chain methanol, such as

methanol or ethanol, with fat or oil (triglyceride) to form glycerol (glycerin) and the esters of long chain fatty acids. Biodiesel is “a substitute for, or an additive to diesel fuel that is derived from the oils and fats of plants and animals”. Increasing uncertainty about global energy production and supply, environmental concerns due to the use of fossil fuels, and the high price of petroleum products are the major reasons to search for alternatives to petro-diesel. Moreover, biodiesel fuel has become more attractive because of its environmental benefits due to the fact that plants and waste temple oils and animal fats are renewable biomass sources. Biodiesel represents a largely closed carbon dioxide cycle (approximately 78%), as it is derived from renewable biomass sources. Compared to petroleum diesel, biodiesel has lower emission of pollutants, it is biodegradable and enhances the engine lubricity and contributes to sustainability. Use of (unprocessed) waste temple oil in the compression ignition engines is reported to cause several problems due to its high viscosity. Biodiesel which is used as an attractive alternative fuel, is prepared by transesterification of waste temple oils with an methanol in presence of a catalyst. The use of waste temple oil as biodiesel feedstock reduces the cost of biodiesel production since the feedstock costs constitutes approximately 70-95% of the overall cost of biodiesel production. Hence, the use of waste temple oil should be given higher priority over the edible oils as biodiesel feedstock. [6] Small amount of biodiesel can be used to add in low sulphur formulation of diesel to increase the lubricating capacity that is lost when sulphur is removed.

1.1 Petrodiesel is an hydrocarbon mixture, considered to be a fuel oil and 18% denser than gasoline. It contains higher quantities of sulphur, reduction in level of sulphur is required in diesel fuels. Higher concentration of sulphur in diesel are harmful for environment. To control the diesel particulate emission, used nitrogen oxide adsorbers to reduce

emissions. For lubrication of engine addition of sulphur is important. Lowering the content of sulphur reduces lubricity of the fuel.

1.2 Advantages of biodiesel

Renewable fuel, obtained from waste temple oils or animal fats. low toxicity, in comparison with diesel fuel degrades more rapidly than diesel fuel, minimizing the environmental consequences of biofuel spills. Lower emissions of contaminants like carbon monoxide, particulate matter, polycyclic aromatic hydrocarbons, aldehydes. It lowers health risk, due to reduced emissions of carcinogenic substances. No sulphur dioxide (SO₂) emissions occur. It may be blended with diesel fuel at any proportion; both fuels may be mixed during the fuel supply to vehicles. Excellent properties as a lubricant. It is the only alternative fuel that can be used in a conventional diesel engine, without modifications. Used cooking oils and fat residues from meat processing may be used as raw materials.

1.3 Use of Biodiesel all over the world

Many international automobile companies have started manufacturing cars that are compatible with biodiesel. These cars run on various blends of biodiesel. The companies include Chevy, Volkswagen, Mercedes-Benzes. Recent news suggests that, various railway trains present in United Kingdom have started to use biodiesel as its fuel source. Disney land has also started employing biodiesel for running of its internal tracts. United States was the first country to run entire flight duration of an airplane solely on biodiesel. This has encouraged other nations to follow suit.

1.4 Source used for biodiesel production

The source of oil used for production of biodiesel is 'waste temple oil' collected from 'Shani' temple from Shani Shingnapur, Taluka. Newasa, District. Ahmednagar, Maharashtra, India having Latitude 19.381°N, Longitude 74.85°E.

1.5 Chemical composition of biodiesel

It consists of alkyl usually methyl esters. It has combustion properties similar to regular diesel. Chemical formula for diesel fuel is C₁₂H₂₆. Biodiesel is derived from waste temple oils such as triglycerides which are esters of glycerol with fatty acids.

Classification of biodiesel: According to the various sources used in the production of biodiesel, the following classes exist:

Primary: Waste temple oil

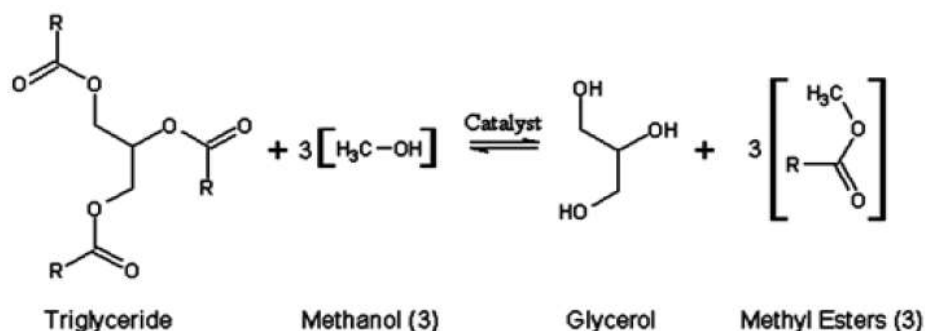
Secondary: Feedstock- sunflower, canola, soya bean

Tertiary: Algae

1.6 CHEMICAL METHOD

Transesterification refers to the reaction of an ester group with an alcohol that has a structure different to that of the original alcohol moiety of the ester, such that a new ester group is formed in which the original alcohol moiety is exchanged with that of the reacting alcohol. In the case of the transesterification of triglycerides of fatty acids (waste temple oil) with methanol (classical/biodiesel production process), the three ester groups of a triglyceride molecule in which three fatty acid moieties are attached to a single glycerol moiety (i.e. glycerol) react with three molecules of methanol to yield three molecules of esters each containing a single fatty acid and methanol moiety and one molecule of glycerol. Therefore, the general chemical name of biodiesel produced by the transesterification of waste temple oil is fatty acid methyl esters. Methanol is commonly used in industrial biodiesel production as a result of its relatively low cost and easy availability. The classical reaction protocol for the transesterification of triglycerides with methanol using homogeneous catalysts such as Sodium methoxide requires mixing and stirring the reagents in a batch reactor. At the end of the reaction, the non-polar phase containing the ester and the polar phase containing glycerol and methanol are separated to recover the products, catalyst, and the excess of methanol.

The transesterification reaction is given below [11].



1.6.1 Parameters affecting biodiesel production:

It is necessary to mention some important process variables of biodiesel production from waste temple oils such as the methanol/oil ratio, reaction time, mixing intensity, temperature and catalysts used.

a. Temperature and time

Typically, the transesterification reaction is complete within around 1 hr using a methanol/oil Molar ratio of 6:1 at a reaction temperature of 60°C.

b. Oil : methanol ratio

The molar ratio of methanol to triglycerides is an important variable that affects the yield of biodiesel in the transesterification reaction. Most systems for transesterification require a methanol/triglyceride molar ratio of 6:1. The excess of methanol with respect to the reaction stoichiometry is needed to shift the reaction equilibrium to the right (product side).

c. Catalyst

The selection of an appropriate catalyst is of fundamental importance for the design of a sustainable transesterification process.

Homogeneous alkaline catalysts, such as sodium hydroxide and potassium hydroxide, are most commonly used in industrial transesterification processes for biodiesel production, mainly because they are able to efficiently promote the reaction at relatively low temperatures. **Heterogeneous catalysts** have the general advantage of being reusable and easy to separate from the reaction products (generally cleaner process). In addition and more specifically, they do not form soaps, are more selective to biodiesel (purer product), and simplify the glycerol purification. A lower methanol-to-oil ratio, easier product recovery, and higher environmental compatibility than chemical catalysts (homogeneous or heterogeneous). Furthermore, fats present in oils/fats can be completely converted into alkyl esters using enzyme catalysts. Notably, new immobilization technology indicates that enzyme catalysts can become cost-effective as compared to chemical processing. However, the production cost of lipases, which is the most investigated and promising class of enzyme catalysts for transesterification, is still significantly higher than that of alkaline catalysts. Enzyme-based technology is still at a stage of intensive research and process optimization.

1.7 BIOLOGICAL METHOD

It has been shown that the enzymatic production of biodiesel is possible by using either extracellular or intracellular lipases. The choice of the method should

be based on the balance between simplified upstream operations (intracellular) and high conversions (extracellular). Both types can be immobilized for use without a need for downstream operations. Moreover, immobilized enzymes are generally more expensive than chemical catalysts. [12]

Domain	Bacteria
Phylum	Proteobacteria
Class	Gamma proteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	<i>Pseudomonas</i>
Species	<i>aeruginosa</i>

Microorganism used- *Pseudomonas aeruginosa*

Immobilization of cells

It is used for intact or disintegrated dead cells that contain active enzymes or resting or growing cells. This technique is used especially with eukaryotic cells where the whole metabolic machinery is often required for their specific application.

1.7.1. Methods of cell immobilization

a) Carrier-free immobilization: Immobilization of a given biomass onto a preformed carrier surface. Immobilization of a given biomass during the course of carrier formation (e.g., by polymerization). Immobilization by controlled growth of an inoculum or by germination of immobilized spores.

b) Alginate: It is extracted from seaweed and is a linear copolymer of *b*-D-mannuronic acid and *a*-L-guluronic acid linked by 1,4-glycosidic bonds. It forms a gel in the presence of multivalent ions, usually calcium or aluminum. The controlled entrapment of cells is simple and generally nontoxic. Various cell types can be immobilized with negligible loss of viability.

Advantages of cell entrapment method:

1. Entrapment is simple and proceeds under very mild conditions.
2. Prepolymers do not contain toxic monomers.
3. The network structure of the gels can be adapted as required.
4. Optimal physicochemical gel properties can be achieved by selecting suitable.

II. MATERIALS AND METHODS

2.1 Collection of waste temple oil:

The waste temple oil was collected in sterile plastic bottle from ShaniShingnapur temple and transported to the laboratory for further study.

2.2 Characteristics of oil and its calculations

a. Specific gravity: -The specific gravity was determined with a specific gravity bottle. The temperature at which the specific gravity was determined 30°C/30°C. The specific gravity was calculated by the formulae:

Specific gravity at 30°C / 30°C = A-B/C-B

where A = weight in g of the specific gravity bottle with oil at 30°C,

B = weight in g of the specific gravity bottle, and

C = weight in g of the specific gravity bottle with water at 30°C

The specific gravity of the fuels at 15.6 °c was also employed to find the specific gravity at other temperatures by using the ATSM D1250 petroleum measurement tables (1953).

b. Viscosity of oil: Viscosity was measured by Oswald viscometer from the following formula

Viscosity (η) = $n_w t_s \cdot d_s / T_w \cdot d_w$

Where η_s = viscosity of the sample in cp at room temperature

η_w = viscosity of the water in cp at room temperature

t_s = time of flow of sample (vol=v) in sec.

t_w = time of flow of water (vol=v) in sec.

d_s = density of sample in g/l at room temperature

d_w = density of water in g/l at room temperature.

c. Acid number: 2 g of oil sample were dissolved in 25 ml of methanol (ethanol) and added 2 to 3 drops of 1 % phenolphthalein indicator, titrated against by 0.1 N NaOH.

Acid number = N EV/W

d. Saponification value: It was also calculated by the formula :

Saponification value = 56.1N/W

where B = volume in ml of standard hydrochloric acid required for the blank

S = volume in ml of standard hydrochloric acid required for the sample

N = normality of the standard hydrochloric acid,

and W = weight in g of the material taken for the test

w g of the sample requires x mg KOH

1 g of the sample requires x/w mg KOH

Results of all characteristics of oil were noted down.

2.3. Methods for biodiesel formation:

2.3.1. Chemical method:

a. Titration

1g NaOH / 1 lit solution in burette was taken. Titration was done in a conical flask, that contained 10ml isopropyl methanol, 1ml waste temple oil, 1-2 drops phenolphthalein indicator with constant shaking. When the solution changes from colorless to faint pink, it indicated end point of the reaction. The burette reading was noted down. Burette reading was divided by 10 and then 3.5 was added to obtain the grams of NaOH which was required for conversion of 1 liter of waste oil temple oil into biodiesel.

b. Pre- treatment of waste temple oil

The oil was filtered to remove debris such as leaves, flowers, dirt etc. Heating of the oil up to 50-60°C, was done to reduce the viscosity.

c. Transesterification reaction

In a bottle NaOH was added to methanol, closed the bottle and shaken well, to dissolve the entire NaOH into methanol. This reaction was exothermic and was accompanied by warming the bottom of the bottle. It was resulted in the formation of sodium methoxide. This mixture was then added to the pre- treated waste temple oil. The flask was kept on a magnetic stirrer for 2 hrs at 500-600 rpm to mix the oil with the Sodium methoxide properly.

d. Separation process

The entire contents of the flask were poured into a separating funnel after 2 hrs. The apparatus was left undisturbed overnight. The next day 2 layers were observed. First layer was Glycerol - the bottom layer (by product) and second layer was biodiesel- the upper layer (honey colour)

Glycerol was poured off in flask and biodiesel was stored separately.

e. Soap formation:

The amount of glycerol formed was measured. It was heated at 65-70°C in a boiling water bath to remove traces of methanol. 1.2g of NaOH was dissolved in 10 ml of warm water and added to the above mixture. Heated the mixture for further 20 min with continuous stirring. Dye was added and mixed properly. The contents were poured into a tray and covered to allow solidification for 2-3 days. It was followed by curing for 4 days to ensure both sides were dried properly before usage.

2.3.2. Biological method

a. Bacterial culture:

Pseudomonas strains were collected from NCIM, Pune during this work. Nutrient agar plates and nutrient agar slants were prepared. The culture was maintained on agar slant and stored at 4°C in refrigerator. Streaking of *Pseudomonas aeruginosa* on the agar plates and slants

was done and incubated for 2-3 days at 25°C. The inoculate was grown aerobically at 25°C in a shaker incubator at 200 rpm. Centrifugation was done at 1200 rpm for 20 minutes and active cells were isolated and further used for immobilization method.

b. Cells immobilization by entrapment method: The sodium alginate entrapment of cells was performed according to the standard method. Alginate solution with a concentration range of 0.5 - 10% was used for the cell immobilization and was prepared by dissolving sodium alginate in boiling water and autoclaved at 121°C for 15 min. Both alginate slurry and cell suspension was mixed and stirred for 10 min to get a uniform mixture the alginate/ cell mixture which was extruded drop by drop into a cold sterile 0.2 M CaCl₂ solution through a sterile 5 ml pipette and kept for curing at 4°C for 1 h. The beads were hardened by resuspending into a fresh chilled CaCl₂ solution for 24 h at 4°C with gentle agitation. Finally these beads were washed with distilled water to remove excess calcium ions and untrapped cells. When the beads are not being used, they are preserved in 0.86% sodium chloride solutions in the refrigerator. Methanolysis was carried out by adding methanol, 3ml hexane and immobilized whole cells to waste temple oil. This mixture was kept in a shaker incubator at 200 rpm for 48 hrs at 25°C. Reaction was then stopped by removing the beads by filtration. The contents were poured in a separating funnel and leaved overnight. Glycerol and biodiesel were separated into two distinct layers and collected biodiesel which was stored separately.

2.3.3. Optimum parameters of transesterification reaction for production of biodiesel.

a. Effect of temperature on the transesterification reaction: Effect of temperature on the transesterification reaction was examined at the temperature range from 50°C to 65°C. Four conical flasks containing 50 ml of waste temple oil, 3 ml hexane, methanol (1:6 molar ratio of oil/methanol) and 3g beads of immobilized cell concentration. These flasks incubated at four different temperatures respectively 50°C, 55°C, 60°C and 65°C at 100 rpm for 48 hrs.

b. Effect of oil / methanol molar ratio on the transesterification reaction: Oil/ methanol molar ratios were effect on the yield of biodiesel, because to shift the transesterification reaction in forward direction. It is necessary to use either an excess amount of methanol or to remove one of the products from the reaction or mixture. Experiments were carried out with different molar ratios of 1:1, 1:2, 1:3 (oil to methanol) at constant levels of 50 ml waste temple oil, 3 ml hexane and 3g immobilized cell concentration in a conical flasks. This

reaction mixture incubated at optimum conditions such as temperature 60°C, pH 7.0 and reaction time 48 h. Fig2 shows that the increasing the molar ratio, the yield of biodiesel was found to be increasing.

2.3.4. Laboratory tests:- Performed for biodiesel obtained by chemical and biological method.

a. Methanol test:

One part of biodiesel was added to 9 parts of methanol by volume. Results were noted down.

b. Firewall test:

Foam formation was observed at the top of test tube after addition of 2ml of biodiesel. Results were observed.

c. Flammability test:

Biodiesel was placed in petri plate and lit fire to it and observed.

d. Clarity test:

2-3 drops of biodiesel obtained was poured on a newspaper. Results were seen.

III. RESULTS AND DISCUSSION



Fig.1: Waste temple oil sample

3.1 Characteristics of waste temple oil value

Saponification number	12.70 mg KOH/g oil
Specific gravity at 30°C	0.91236
Viscosity of oil	49.520 mm ² /s at 303 K
Acid number (with ethanol)	9.7 mg KOH/g oil

3.2 Chemical method for Biodiesel Production

Biodiesel was obtained by chemical method using transesterification reaction. After separation process, Biodiesel and Glycerol were obtained.

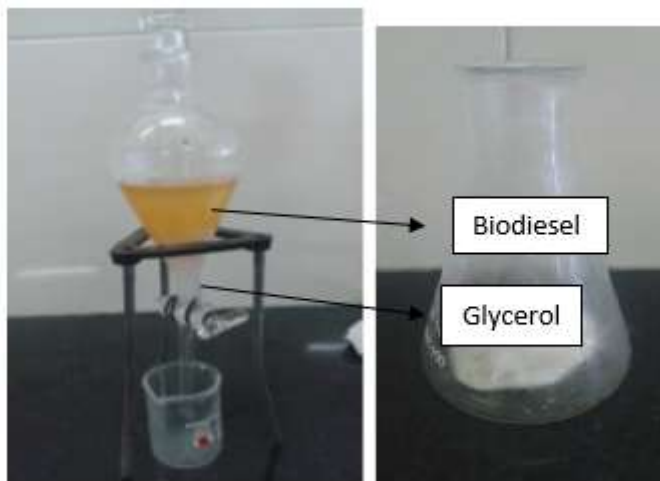


Fig.2: Biodiesel obtained by chemical method Fig.3: Glycerol obtained by Chemical method

Using glycerol obtained from separation process, soap was formed.



Fig.4: Formation of Soap

3.3 Biological method of biodiesel formation:

In present work experiments were carried out to growth of culture, *Pseudomonasaeruginosa* produced from NCIM,Pune and maintained it on agar slant, cell immobilized by entrapment method.



Fig.5: Immobilized *Pseudomonasaeruginosa* beads by calcium alginate method

3.4 Effect of temperature on transesterification reaction-

The highest percentage of conversion of oil into biodiesel was observed at 60°C and thereafter decreases due to denature of the enzyme. Biodiesel yield found to be 88%.

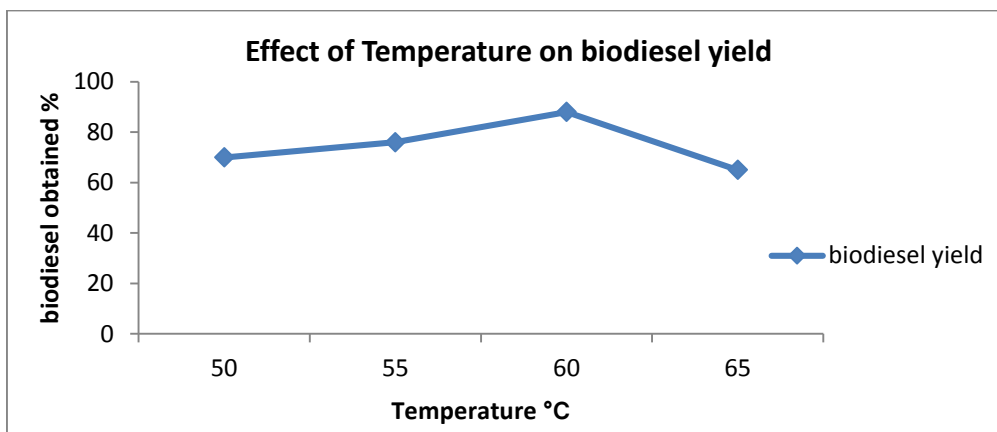


Fig.7: Effect of temperature on biodiesel yield

3.5 Effect of oil/methanol molar ratio on transesterification reaction-Maximum yield were obtained at molar ratio of 1:2. Further yield of

biodiesel was found to be decreasing with increasing molar ratio beyond 1:2. It was due to the inhibition of excess methanol reduces the enzyme activity.

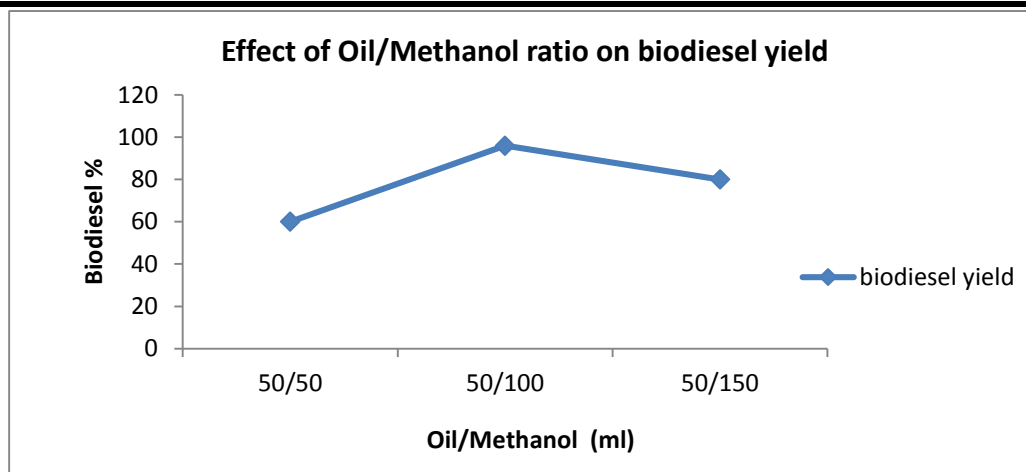


Fig.8: Effect of Oil/Methanol on biodiesel yield

From the above data one concludes that 1:2 ratio of Oil: Methanol is best suited for maximum biodiesel yield which was obtained 96%.

3.6 Laboratory test:

Test	Result
Methanol test	No precipitation
Firewall test	Impure with glycerol
Flammability test	Flammable
Clarity test	No debris in biodiesel

Methanol Test- Since no precipitation was formed it indicated that biodiesel obtained was not converted into any other product.

Firewall Test- This indicated that the biodiesel obtained was not 100% pure and contained small amount of glycerol contamination.

Flammability Test- Resulted in combustion. This was achieved to prove the flammability property of obtained biodiesel.

Clarity Test- Since the newspaper could be read easily it satisfied the visibility or clarity test. It also indicated that there were no debris present in the obtained biodiesel.

toxicity in comparison with diesel fuel, degrades more rapidly minimizing the environmental consequences of biodiesel spills.

V. CONFLICT OF INTEREST

We all authors do not have any conflict of interest.

VI. ACKNOWLEDGEMENT

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IV. DISCUSSION

Biodiesel can be obtained by chemical method and biological method. Transesterification was carried out using waste temple oil and methanol. Immobilized beads were obtained by cell entrapment method. Characteristics of waste temple oil were studied. Conversion of oil to biodiesel by transesterification reaction and its effects like temperature, oil : methanol ratio on the production of biodiesel yield were obtained 88% and 96% respectively. Whereas, Devendra Pratap Singh, Hemant Kumar (2013) got 76% biodiesel yield from Jatropha oil. Using Soxhelt extraction Bobade S.N and Khyade V. B (2012) obtained 31% yield of *Pongamiapinnataseeds*. One of the Biodiesel byproduct soap was formed. Laboratory tests such as methanol, firewall, flammability and clarity tests were performed to analyze the quality of biodiesel. It is renewable alternative fuel which has low

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