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Production of biodiesel from edible and non-edible oils using *Rhizopus oryzae* and *Aspergillus niger*

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ABSTRACT

Biodiesel fuel (BDF) produced by alcoholysis of vegetable oils or fats is viewed as a promising renewable fuel source. Diminishing petroleum reserves and increasing environmental regulations have made the search for renewable fuel. Biodiesel is non-toxic and biodegradable produced from renewable sources and contributes a minimal amount of net green-house gases, such as CO_2, SO_2 and NO emissions to the atmosphere. The main objective of the present study is to produce biodiesel from vegetable oils (edible and non-edible oil) and to use micro-emulsions with solvents ethanol and methanol following acid, alkali and fungal enzyme catalysis methods. The best suited method of biodiesel production was ethanolic and alkali mediated transesterification process rather than methanolic and acidic transesterification. The maximum yield of biodiesel was obtained from Rhizopus oryzae lipase enzyme, ethanolic and alkali mediated transesterification followed by Aspergillus niger.

Keywords: Biodiesel, transesterification, vegetable oil, *R.oryzae* and *A.niger*

INTRODUCTION

The need of energy is increasing continuously due to rapid increase in the number of industries and vehicles owing to population explosion. The sources of this energy are petroleum, natural gas coal, hydrocarbon and nuclear. The major disadvantages of using petroleum based fuels are atmospheric pollution created by the use of petroleum diesel. The petroleum diesel combustion emits several greenhouse gases. Apart from these emissions, petroleum diesel is also major source of these air containments including NO_X, SO_X, CO, particulate matter and volatile organic compounds. Recently, there are several concerns about the dependence on foreign controlled fuel sources to finite supply, reserves of petroleum shortage and rinsing price of petroleum based fuels. These problems have encouraged the development of alternative fuels obtained especially from renewable sources [1].

Biodiesel refers to a vegetable oil or animal fat based diesel fuel consisting of long chain alkyl (methyl, propyl and ethyl) esters. Biodiesel is typically made by chemically reacting lipids (e.g., vegetable oil, animal fat with an alcohol producing fatty acid esters. Biodiesel is meant to be used in standard diesel engines and is this distinct from the vegetable and waste oils used to fuel converted diesel engines. Biodiesel can be used alone or blended with petro-diesel. Biodiesel can also be used as a carbon alternative to heating oil. The National Biodiesel Board (USA) also has a technical definition of "biodiesel" as a mono-alkyl ester [2,3].

Research has been made to produce biodiesel by using alternative or greener oil resources like edible oils and nonedible oils. The edible oils such as, *Theobroma cacao* (palm oil), *Cocos nucifera* (coconut oil), *Helianthus annus L*. (sunflower oil) and *Sesamum indicum* (gingelly oil) and the non-edible oils includes *Ricinus communis*(caster oil), *Azadiracta indica* (neem oil), *Jatropha curcas* (jatropha oil) and *Prunus dulcis* (almond oil) were found to be suitable for biodiesel production under the experimental conditions. [16] found that the yield of methyl ester from karanja oil under the optimal condition was 97-98%. Oil content in the Caster bean, Hemp and Pongame seed was around 50, 35 and 30-40% respectively. Neem seed contains 30% oil content. Biodiesel was the pure or 100%, biodiesel fuel [4].

The recent discovery of a variant of the fungus *Rhizopus oryzae* and *Aspergillus niger* points toward the production of so called myco-biodiesel or fungal biodiesel from cellulose. This organisms were recently discovered in the rainforests of northern Patagonia and has the unique capability of converting cellulose into medium length hydrocarbons typically found in diesel. The present study was undertaken to collect and analyse the physico-chemical characteristic of soil, isolate and identify the test organisms, study the transesterification reaction of vegetable oils, production and analysis of biodiesel characteristics.

MATERIALS AND METHODS

Oil material collection

Commercially available edible and nonedible oils were purchased from local grocery shop. The edible oils obtained from the seed *Theobroma cacao* (palm), *Cocos nucifera* (coconut), *Helianthus annus L.* (sunflower), *Sesamum indicum* (gingelly) and the non-edible oils obtained from the seed *Ricinuscommunis* (caster oil), *Azadirachta indica* (neem oil), *Jatropha curcas* (jatropha oil) and *Prunus dulcis* (almond oil). Oils were subjected to analyse fatty acid composition [9].

Analysis of physico-chemical properties of soil

Soil sample collected from Sengamala Thayaar Educational Trust Women's college playground, Mannargudi. The collected soil sample was subjected to analyse physico-chemical properties [17].

Isolation and identification of test organism

The soil sample was serially diluted up to 10^{-1} to 10^{-7} and the dilution poured on the PDA plates. Then the plates were incubated. After incubation, the colonies were identified by using microscope [10].

Transesterification of vegetable oils

The liquid medium was centrifuged at 10,000 rpm for 10 minutes and then the supernatant was discarded. The pellet was taken in 5 ml of methanol: chloroform in 2:1 ratio and kept in shaker for 20 minutes, then centrifuged at 10,000 rpm for 10 minutes. The organic phase was washed with one ml of water and again centrifuged at 20,000 rpm for 5 minutes. The upper aqueous phase was removed and the lower organic phase was rinsed twice with 5ml of methanol and water in 1:1 ratio. Finally, the extracted lipid with lipase was collected from the solvent phase and stored for further experimental work [5].

Production of biodiesel

Transesterification reaction process also called alcoholises, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis except than an alcohol is used instead of water [5, 6]. This has been widely used to reduce the viscosity of the triglycerides. The transesterification is represented as:

$$\underset{\text{ester}}{\text{R-COO-R}} + \underset{\text{alcohol}}{\text{R-OH}} \longleftrightarrow \underset{\text{new ester}}{\text{R-COO-R}} + \underset{\text{alcohol}}{\text{R-OH}}$$

The transesterification reaction was performed by combining edible and non-edible oil with alcohol or methanol in the presence of a catalyst Sodium hydroxide or Hydrochloric acid and fungal enzyme. The esterification mixture consisted of 100 ml of oil, 20 ml of ethanol or methanol, 3g of NaOH or 3 ml HCl and 5ml of fungal enzyme. The experiment was performed at 40˚C and the reaction time was kept constant for 3 hours for all the experiments.After the transesterification reaction, the produced biodiesel was separated from glycerol with the help of separating funnel and finally washed with 5% water followed by Magnesium sulphate anhydrous to remove the water. The biodiesel and glycerol ratio was recorded [6].

Analysis of biodiesel

After separation of the two layers, the upper layer of biodiesel was purified by distilling the residual methanol at 60˚C. The remaining catalyst was removed by successive rinsing with distilled water by adding 1-2 drops of acetic acid to neutralize the catalyst. The residual materials can be eliminated by treatment with anhydrous Sodium sulphate ($NaSO_4$) followed by filtration. Transparent blackish liquid was obtained as the final product. Biodiesel analysis such as viscosity, density and flash point were analysed [7]. High viscosity is the major problem preventing the use of vegetable oil directly in diesel engines as it affects the flow of fuel and spray characteristics [8].

RESULTS AND DISCUSSION

Analysis of vegetable oils

The fatty acid composition of the edible and non-edible oils were used for biodiesel production revealed the presence of butyric, caprolic, caprylic, capric, lauric, myristic, palmitic, steric, oleic, linoleic, α-linoleic, erucic, EPA, eicosenic, riconoleic and dihydrosteric acids identified as per the peak position and the relative retention time of those standard methyl esters in TLC chromatograph. The investigation of the fatty acid composition of the vegetable oils by Thin Layer Chromatography (TLC) [9].

Isolation and identification of test organisms

In this study, Sengamala Thayaar Educational Trust Women's college playground soil was collected, analysed the physico-chemical properties and presented in the table 1. The sample was used for the isolation of the fungal species using serial dilution and plating methods. Serially diluted sample was poured into the Potato Dextrose Agar medium (PDA) showed the number of fungal species. The colonies were identified by lacto phenol cotton blue method using manual of soil fungi. The following two species were isolated and identified viz, *Aspergillus niger* and *Rhizopus oryzae*.

Biodiesel production

Edible oil biodiesel such as, POB, GOB, COB and SOB yield 25.64 ml, 31.1 ml, 24.65 ml and 20.22 ml respectively were obtained in 100ml of edible oil with ethanol, NaOH and *Rhizopus oryzae* lipase mediated transesterification process. This was followed by *Aspergillus niger* lipase mediated transesterification process. The ethanol NaOH or ethanol-NaOH fungal lipase mediated transesterification process in all the reactions produced lesser quantities of biodiesel such as POB, GOB, COB and SOB i.e., 21.83 ml, 28.27 ml, 21.4 ml and 17.03 ml respectively presented in table 1.

The maximum yield of non-edible oil biodiesel such as JOB, AOB, ROB and NOB i.e., 58.13 ml, 45.60 ml, 43.3 ml and 59 ml respectively were obtained in 100 ml of non-edible oil with ethanol, NaOH and *Rhizopus oryzae* lipase mediated transesterification process. This was followed by *Aspergillus niger* lipase mediated transesterification process. The methanol – NaOH fungal lipase transesterification process in all the reactions produced lesser quantities of biodiesel such as JOB, AOB, ROB and NOB i.e., 44.95 ml, 40.55 ml, 40 ml and 51.33 ml respectively presented in table 2.Our findings similar to the yield of methyl esters of HOB, NOB, POB and COB were investigated by changing catalyst concentrations while molar ratio (6:1) methanol/oil and temperature (60˚C) was kept constant. The highest conversion rate was obtained with the catalyst concentration of 0.7g, under these conditions the biodiesel yield was 74.36%, 70%, 77.16% and 70% for HOB, NOB, POB and COB respectively [10, 5 and 4].

Analysis of biodiesel

The experimental investigation was carried out for different fuel properties and the performance was evaluated according to ASTM D-445, D-1298, D-93, D-1500and D-4294 compared with diesel.Our findings similar to High Speed Diesel (HSD) have viscosity of 1.3 - 4.1 @40˚C whereas the viscosities of COB and POB are 5.67 and 5.5 respectively which was slightly higher than the viscosity of HSD presented in table 3. While the viscosities of HOB and NOB were 3.83 and 4.81 which were closer to HSD. It shows that viscosities of these non-edible oil seeds biodiesel were comparable to HSD [11, 12, 4, 13 and 14].

Table-1 Physico-chemical characteristics of the soil sample

S .No	Properties	Amount
1	pH	6.3
2	Carbon di oxide	30±00 (Kg/ha)
3	Alkalinity	13.0 ± 1.5 (Kg/ha)
4	Nitrate	17.6 ± 1.0 (Kg/ha)
5	Ammonia	25.0 ± 6.8 (Kg/ha)
6	Phosphate	19.2 ± 1.0 (Kg/ha)
7	Calcium	13.9 ± 0.7 (Kg/ha)
8	Chloride	11.5 ± 0.5 (Kg/ha)
9	BOD	48.4 ± 1.5 (Kg/ha)

BOD – Biological Oxygen Demand
Values are expressed as Mean ±Standard Deviation

Table No.2 Biodiesel yield of edible oils

S .No	Plant Oil Biodiesel	Concentration of catalyst NaOH(g)	Amount of catalyst NaOH(g)	*Rhizopus oryzae*		*Aspergillus niger*	
				Biodiesel (%)	Glycerine (%)	Biodiesel (%)	Glycerine (%)
1	POB	Normal	0.7	48.83	51.17	40.48	59.52
		Double	1.4	22.21	77.79	20.21	79.79
		Half	0.35	5.89	94.11	4.80	95.2
2	GOB	Normal	0.7	53.41	46.59	50.84	49.16
		Double	1.4	30.48	69.52	26.48	73.52
		Half	0.35	9.41	90.59	7.49	92.51
3	COB	Normal	0.7	45.21	54.79	40	60
		Double	1.4	20.42	79.58	18.90	81.1
		Half	0.35	8.30	91.70	5.3	94.7
4	SOB	Normal	0.7	38.83	61.17	35.38	61.62
		Double	1.4	15.41	84.59	10.49	89.51
		Half	0.35	6.42	93.58	5.21	94.79

(POB - Palm Oil Biodiesel, GOB - Gingelly Oil Biodiesel, COB-Coconut Oil Biodiesel, SOB - Sunflower Oil Biodiesel

Table No.3 Biodiesel yields of non-edible oils

S .No	Plant Oil Biodiesel	Concentration of catalyst NaOH(g)	Amount of catalyst NaOH(g)	*Rhizopus oryzae*		*Aspergillus niger*	
				Biodiesel (%)	Glycerine (%)	Biodiesel (%)	Glycerine (%)
1	JOB	Normal	0.7	78.36	21.64	63.21	36.79
		Double	1.4	67.62	32.38	52.08	67.62
		Half	0.35	22.42	77.58	18.81	81.19
2	AOB	Normal	0.7	68.12	31.88	62.48	37.52
		Double	1.4	40.26	59.74	38.29	61.71
		Half	0.35	28.41	71.59	20.89	79.11
3	ROB	Normal	0.7	70	30	65	35
		Double	1.4	60	40	55	45
		Half	0.35	00	100	00	100
4	NOB	Normal	0.7	70	30	63	37
		Double	1.4	68	32	55	45
		Half	0.35	40	60	36	64

(JOB - Jatropha Oil Biodiesel , AOB - Almond Oil Biodiesel, ROB - Ricinus Oil Biodiesel, NOB - Neem Oil Biodiesel)

Table No.4 Comparative analysis for fuel properties of edible and non-edible oil biodiesel

S.No	Type of diesel	Viscosity @40˚C cst	Density @15˚C Kg/L	Flash point ˚C
1	JOB	5.67	0.86	122
2	AOB	5.09	0.82	126
3	ROB	5.83	0.88	120
4	NOB	4.81	0.87	124
5	POB	5.09	0.83	120
6	GOB	5.0	0.80	122
7	COB	5.13	0.84	128
8	SOB	5.36	0.82	123
9	HSD	1.3-4.1	0.83	60-80

JOB - Jatropha Oil Biodiesel, AOB - Almond Oil Biodiesel, ROB - Ricinus Oil Biodiesel, NOB - Neem Oil Biodiesel, POB - Palm Oil Biodiesel, GOB - Gingelly Oil Biodiesel, COB-Coconut Oil Biodiesel, SOB - Sunflower Oil Biodiesel, HSD - High Speed Diesel

CONCLUSION

The biggest advantages of biodiesel were environmentally friendliness that has over gasoline and petroleum diesel. The advantages of biodiesel as a diesel fuel were its probability, ready availability, renewability, higher combustion efficiency and higher biodegradability. It's potential for reducing a given economy's dependency on imported petroleum biodegradability, high flash point and inherent lubricates in the heat form. Hence, the main objective of the study was to produce biodiesel from edible and non-edible oils and to use micro-emulsions with solvents ethanol and fungal enzyme catalytic method.

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