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Efficacy of Fungal Enzyme in Biodiesel Production from Vegetable Oil

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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 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 trans-esterification process rather than methanolic and acidic trans-esterification. The maximum yield of biodiesel was obtained from *Rhizopus oryzae* lipase enzyme, ethanolic and alkali mediated trans-esterification followed by *Aspergillus niger, Polyporus squamosus* and *Agaricus campestris.*

Key words: Biodiesel, trans-esterification, Fungal enzyme, Lipase.

The consumption and demand for petroleum products are increasing every year due to increase in population, standard of living and urbanization. Due to gradual depletion of world petroleum reserves and the impact of environmental pollution, there is an urgent need for suitable alternative fuels for use in diesel engines (Kloptenstem, 1988; Harrington, 1986). Today's diesel engines require a clean burning and stable fuel that performs well under a variety of operating conditions. Biodiesel is the only alternative fuel that can be used directly in any existing unmodified diesel engine. Because it has similar properties to diesel fuel, biodiesel can be blended in any ratio with diesel fuel (Masjuki, 1993).

There are many reasons that justify the development of biodiesel as biofuel. It provides a market for excess production of vegetable oils, it decreases the dependence on imported petroleum, it does not contribute to global warming due to its closed carbon cycle, the exhaust emissions of carbon monoxide, unburned hydrocarbons and particulate emissions from biodiesel are lower than regular diesel fuel, when blended with added to crude oil derived diesel fuel upto 20% (Ayhau, 2009).

In view of this, vegetable oil is a promising alternative biofuel, which can be converted into biodiesel and it is produced easily in rural areas, where there is an acute need for modern forms of

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energy (LePori *et al*., 1992; Rao and Gopalakrishnan, 1991). In recent years systematic efforts have been made by several research workers to use vegetable oils as fuel in engines (Masjuki and Sohif, 1991; Nag and Bhattacharrya, 1995; Takeda, 1982; Piyaporn *et al*., 1996). Various oils like algal oil, sunflower oil, palm oil and olive oil have been used in different countries as raw materials for biodiesel production owing to its availability (Dorado *et al*., 2003; Hossain *et al*., 2008; Hossain and Boyce, 2009a, b). Biodiesel is defined as a fuel comprised of monoalkyl esters of long chain fatty acids derived from vegetable oils or animal fats (Vicente *et al*., 2007).

The high viscosity and poor volatility are the major limitations of vegetable oils for their utilization as fuel in diesel engines. High viscosity of vegetable oils deteriorate the atomization, evaporation and air-fuel mixture formation characteristics leading to improper combustion and higher smoke emission. Moreover this high viscosity generates operational problems like difficulty in engine starting, unreliable ignition and deterioration in thermal efficiency. Converting to biodiesel is one of the options to reduce the viscosity of vegetable oils (Pangazhabadivu and Jeyachandran, 2005). For this purpose fungal enzymes are used for trans-esterification process (Jin *et al*., 2008).

The first objective of this study aims to compare the optimum conditions of biodiesel production from commercial oil used for lighting lamps through trans-esterification process using alkaline and acidic catalysts. The second objective is to compare the efficacy of fungal enzyme through alkaline and acidic based trans-esterification process.

MATERIAL AND METHODS

Materials - Oil

Commercially available 'Deepam oil' used for lighting lamps was purchased from a local grocery shop. The oil is a mixture of oils obtained from the seeds of *Azadirachta indica* A. Juss (neem), Meliaceae; *Madhuca longifolia* L. (Mowra - fat) Sapotaceae; *Ricinus communis* L. (Castor oil), Euphorbiaceae and *Sesamum indicum* L. (Gingelly oil), Pedaliaceae in equal proportions by volume.

GLC - Chromatograph of Standard Fatty Acid Methyl Ester Mixture of Commercial 'Deepam Oil' (Vegetable Oil)

The fatty acid composition of the vegetable oil used for this study was investigated by GLC (Gas Liquid Chromatography) after conversion of the acids into the corresponding methyl esters as described by Mangold and Kammereck (1961) and Loury (1967). The converted sample was injected into the column filled with 10% diethyl glycol succinate (DEGS) on 100-200 (British - Std. Sieve) mesh. The injector temperature was 230° C and the detector temperature was 250° C. Nitrogen gas was used as the carrier gas at a flow rate of 11.3 ml/min. Standard methyl esters peaks were identified with Sigma standards.

Fungi

The lipase enzyme was obtained from four different fungi *viz. Agaricus campestris, Aspergillus niger, Polyporus squamosus* and *Rhizopus oryzae. A. niger* and *R. oryzae* cultures were obtained from the Microbiology Lab, National College, Tiruchirappalli and *A. campestris* and *P. squamosus* were collected from natural source in College campus.

Fungal Lipase Extraction

Fungal lipase extraction was carried out according to Folch *et al*. (1957) method. The fungal mycelia in liquid medium or tissue was centrifuged at 10,000 rpm for 10 min. and the supernatant was discarded. The pellet was taken in 5.0 ml of methanol : chloroform in 2 : 1 ratio and kept in shaker for 20 min. then centrifuged at 10,000 rpm for 10 min. The organic phase was washed in 1 ml of water and again centrifuged at 2000 rpm for 5 min. The upper aqueous phase was removed and the lower organic phase was rinsed twice with 5.0 ml 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 and part of the lipase was crystallized and used further (Fig. 1).

Trans-esterification Reaction

Trans-esterification reaction process also called alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis except that an alcohol is used instead of water (Murugesan *et al*., 2009). This has been widely used to reduce the viscosity of the

triglycerides. The trans-esterification is represented as:

$$\underset{\text{Ester}}{RCOOR'} + \underset{\text{Alcohol}}{R''OH} \rightleftharpoons \underset{\text{New Ester}}{RCOOR''} + \underset{\text{Alcohol}}{R'OH}$$

The trans-esterification reaction was performed by combining Deepam 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 Deepam oil, 20 ml of ethanol or methanol, 3 g of NaOH or 3 ml of HCl and 5 ml 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 trans-esterification reaction the biodiesel produced was separated from glycerol using separating funnel and finally washed with 5% water followed by magnesium sulfate anhydrous to remove the water. The biodiesel : glycerol ratio was recorded.

Biodiesel Analysis

Parameters like viscosity, acid number, carbon residue were analysed (Kalam and Masjuke, 2002) and compared with American Standard for Biodiesel Testing method (ASTMD 6751).

RESULTS AND DISCUSSION

GLC - Chromatograph Studies of Vegetable Oil

The fatty acid composition of the vegetable oil used for biodiesel production revealed the presence of capric, caprylic, lauric, myristic, oleic, palmitic, nonanoic, steraric, undecanoic, arachidic and behenic acids identified as per the peak position and relative retention time of those standard methyl esters in GLC-Chromatograph.

Biodiesel Production

The results of biodiesel production by various combinations of esters, catalyst and fungal lipase enzyme mediated trans-esterification process are presented in Table-1.

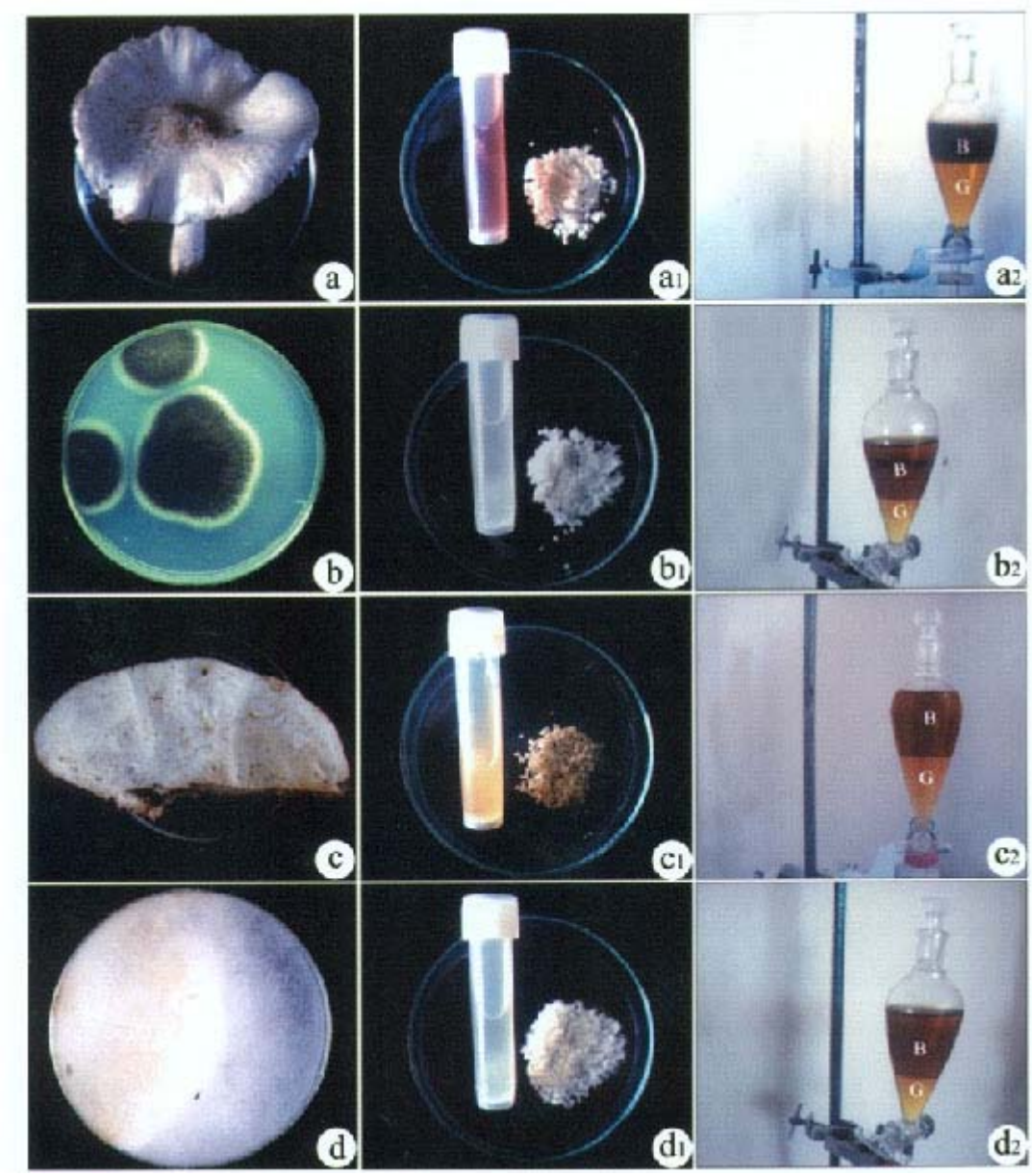
Efficacy of fungal enzyme in Biodiesel Production

The maximum yield of 70 ml of biodiesel was obtained in 100 ml of oil with ethanol, NaOH and *R. oryzae* lipase mediated trans-esterification process. This is followed by *A. niger* (65 ml), *P. squamosus* (55 ml) and *A. campestris* (50 ml) lipase mediated trans-esterification process. The methanol-NaOH or methanol-NaOH-fungal lipase trans-esterification process in all the reactions produced lesser quantities of biodiesel (Table 1 and Fig. 1).

Komers *et al*. (2001) obtained biodiesel from rapeseed oil using methanol and KOH as catalyst. Mittelbach (1993) have produced biodiesel from vegetable oils. Enciner *et al*. (2002) produced biodiesel from vegetable oil of *Cynara cardunculus*. Mohamed and Ali (2002) produced biodiesel from palm oil. Zhang *et al*. (2003), Oliveira and Rosa (2006), Aranda *et al*. (2007), Kalam and Madjuki (2002), Demirbas (2007) and Hossain and Boyee (2009a) used sunflower oil and used cooking oil and produced biodiesel. All these results have shown variations in the output of biodiesel production from the present investigation. The variations are due to the triglyceride content of

Table 1. Biodiesel production at 40° C in 3 hours

Oil (ml)	Esters (ml)	Catalyst (ml or g)	Fungal lipase (ml)	Biodiesel (ml)	Glycerol (ml)
Vegetable oil (100)	Ethanol (20)	NaOH (3)	–	40	60
Vegetable oil (100)	Methanol (20)	NaOH (3)	–	40	60
Vegetable oil (100)	Ethanol (20)	HCl (3)	–	35	65
Vegetable oil (100)	Methanol (20)	HCl (3)	–	35	65
Vegetable oil (100)	Ethanol (20)	NaOH (3)	*A. campestris* (5)	50	50
Vegetable oil (100)	Methanol (20)	NaOH (3)	*A. campestris* (5)	50	50
Vegetable oil (100)	Ethanol (20)	NaOH (3)	*A. niger* (5)	65	35
Vegetable oil (100)	Methanol (20)	NaOH (3)	*A. niger* (5)	60	40
Vegetable oil (100)	Ethanol (20)	NaOH (3)	*P. squamosus* (5)	55	45
Vegetable oil (100)	Methanol (20)	NaOH (3)	*P. squamosus* (5)	50	50
Vegetable oil (100)	Ethanol (20)	NaOH (3)	*R. oryzae* (5)	70	30
Vegetable oil (100)	Methanol (20)	NaOH (3)	*R. oryzae* (5)	65	35



a) *Agaricus campestris*, a1) Enzyme & Crystals, a2) biodiesel production
b) *Aspergillus niger*, b1) Enzyme & Crystals, b2) biodiesel production
c) *Polyporus squamosus*, c1) Enzyme & Crystals, c2) biodiesel production
d) *Rhizopus oryzae* d1) Enzyme & Crystals, d2) biodiesel production
B) Biodiesel G) Glycerin

the oil, the type of fungal lipase and the type of transmethylation reaction during the production of biodiesel.

Pazonki *et al.* (2010), Kim *et al.* (2007) and Jin *et al*. (2008) used whole cell as well as *Rhizopus oryzae* and *Candida rugosa* lipase as biocatalysts in biodiesel-fuel production. The fungal lipase proved vital in catalylic activity of esterification process and generated high quality and quantity of biodiesel as reported by Kloptenstem (1988).

Biodiesel Analysis

Biodiesel analysis such as viscosity, acid number and carbon residue revealed 4.60, 0.20 and 0.021 respectively as against ASTMD 651, 1.9-6.0 mm^2/sec at 4° C for viscosity, 0.5 mg KOH/g ASTMD 651 for acid number and < 0.3 EN 14214 for carbon residue.

CONCLUSION

The optimum conditions for biodiesel - fuel production from Deepam Oil showed that the oil may be employed as a substantial source of biodiesel as fuel in diesel engines. This research represented that the production of biodiesel from ethanol or methanol and catalyst NaOH or HCl has shown no significant differences in the biodiesel yield. However ethanol, NaOH and *R. oryzae* or *A. niger* lipase yielded significant quantity of biodiesel fuel which is considered as renewable energy.

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