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Lipase catalysed biodiesel production by transesterification of oil extracted from seeds of *Madhuca indica*

• **Maitri Jha** • **Damini Skandhwasini** • **Jahanvi**
• **Urvashi Sinha**

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Corresponding Author : Urvashi Sinha

Abstract : *In this study, extracted seed oil of Madhuca indica was used for production of biodiesel through the process of lipase catalysed transesterification. The lipase enzyme was extracted from the fungal source Rhizopus spp. The use of lipase in the biodiesel production proved an efficient method for increasing its yield and in providing environment friendly "Green reaction". The fuel produced by the seed oil showed similarities to the petrodiesel as thre density and viscosity of biodiesel was found to be 872 kg/l and 4.39 l/s respectively which was almost similar to petrodiesel. This fuel produced is non toxic,renewable and biodegradable. The yield of biodiesel was influenced by the concentration of methanol added to oil. From the study, it was clear that the properties of biodiesel produced from oil of Madhuca indica, can be effective alternative to diesel fuels and other such depleting fossil fuels.*

Keywords: *Biodiesel, Lipase, Madhuca indica, Transesterification.*

Maitri Jha
B.Sc. III year, Botany (Hons.),
Session : 2016-2019, Patna Women's College,
Patna University, Patna, Bihar, India

Damini Skandhwasini
B.Sc. III year, Botany (Hons.),
Session : 2016-2019, Patna Women's College,
Patna University, Patna, Bihar, India

Jahanvi
B.Sc. III year, Botany (Hons.),
Session : 2016-2019, Patna Women's College,
Patna University, Patna, Bihar, India

Urvashi Sinha
Asst. Prof., Department of Botany,
Patna Women's College, Bailey Road,
Patna – 800 001, Bihar, India.
E-mail : .urvashi_vrm@yahoo.co.in

Introduction :

Lipase catalysed transesterification of *Madhuca indica* oil has been considered as one of the most promising techniques for providing biodiesel. *Madhuca indica* of family Sapotaceae is native to North-eastern regions of India like Bihar, Madhya Pradesh, Uttar Pradesh, Jharkhand etc. Due to its large population and phenomenal growth rate it is easily available in all regions and thus further can be used for oil extraction and its biodiesel formation. *Madhuca indica* oil seeds also contain free fatty acid content upto 18% and are considered as a good source of biodiesel (Rajendran et al 2016). Biodiesel is non-petroleum based diesel fuel consisting of mixture of fatty acid alkyl ester. The process of transesterification transform the the higher chain of fatty acid into simpler one. Since, conventional method adopts

transesterification using chemical catalyst and only few have employed enzymatic approach for industrial application. This is mainly because of high expense of catalyst required for biodiesel production from plants. Lipase can easily be isolated from microbial source, fungi like *Rhizopus.* The source of extraction is extracellular and involves simple steps as reported by Pogori et al (2007). Hence, the use of *Madhuca indica* oil as diesel substitute exhibits greater importance in the field of fuel currency.

Materials and Methods :

The seeds of *Madhuca indica* were collected from local area of Patna for oil extraction. The fungal strain of *Rhizopus* was isolated from citrus fruit for extraction of lipase enzyme.

About 250grams of *Madhuca indica* were collected from local area of Patna. Seeds were washed properly and kernels were separated from seed. Separated kernels were dried in hot air oven at 45° C for 2-3 days. Dried seed samples were grinded well and oil was extracted through cold pressing technique which was about 10mL (Atabani et al 2013).

In next step for the purpose of lipase extraction the fungal strain of *Rhizopus* was isolated from citrus fruit like pear and a culture media was prepared using Potato Dextrose Agar media for proper growth of *Rhizopus*. The culture media was incubated at 28° C for 7 days.

Potato culture broth was prepared for liquid media of *Rhizopus* which was centrifuged at 6000rpm at 4° C for 40 minutes and subjected to ice cold acetone for precipitation. This resulted in the formation of lipase pellet which was vortexed with 20 millimolar Tris-HCl buffer at pH equals to 7.2 for the extraction of enzyme. This homogeneous mixture so formed is actually lipase extract (Pogori et al 2007).

Lipase extract was subjected to volumetric titration method, against 0.05N $KMnO_4$ solution for estimation of its enzymatic activity as reported earlier by Vijaylakshmi et al (2012) using formula:

$$\text{Units/mL of enzyme} = \frac{\text{Final burettereading x Normality of } KMnO^4 \text{ x Time of incubation x Dilution factor}}{\text{Volume of enzyme added}}$$

Madhuca indica oil and ethanol in the presence of lipase extract for transesterification are mixed in conical flask and placed inside shaker incubator at 40°-50° C and 200rpm for 8-16 hours as reported earlier by Vijaylaksmi et al (2012). Incontinuity of agitation affects the biodiesel yield. But the effect was ignored in present study. A general reaction involved in lipase catalysed transesterification is depicted in (Fig. 1).

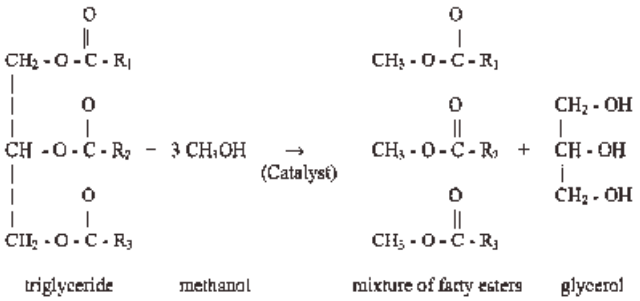

Fig. 1. Transesterification reaction of triacylglycerol into esters of fatty acid and glycerol

After completion of transesterification, biodiesel was formed along with its byproduct glycerol. Further biodiesel and glycerol was separated out using separating funnel.

Results and Discussion :

Results of the physical parameters of the oil extracted from *Madhuca indica*, its biodiesel and petrodiesel were estimated and tabulated (Table 1).

Table 1. Estimation of physical parameter of oil extracted from *Madhuca indica,* its biodiesel and petrodiesel

S. No.	Type of fuel→ Property ↓	Unit	Madhuca indica oil	Madhuca indica biodiesel	Petrodiesel
1.	Density	kg/m^3	915	872	839
2.	Viscosity	m^2/s	24.58	3.9	3.18
3.	Solubility Appearance in Alcohol	–	Partially soluble	Soluble and gives cloudy appearance	Soluble and gives cloudy appearance

Both density and viscosity of oil was decreased after transesterification due to transformation of higher chain alkyl fatty acid into smaller ones. Density and viscosity of biodiesel are almost similar to petrodiesel. This result was in good agreement with Srivastav et al (2015) and Alamu et al (2008).

The solubility of *Madhuca indica* oil was partially soluble in alcohol while its biodiesel as well as petrodiesel were completely soluble with formation of cloudy appearance for short interval of time (Kwanchareon et al 2007).

Lipase activity estimated through volumetric titration was 0.0125 enzyme unit/mL.

Nature of reaction mixture was transformed from heterogeneous solution to homogeneous solution after transesterification because lipid was insoluble in water but water is essential to use in mixture for the activation of lipase.

As the concentration of methanol increases, the yield of biodiesel also increases. However, as the concentration of methanol increases very high, the yield of biodiesel sequentially decreases (Fig. 2) similar finding was reported by Arumugam et al (2017).

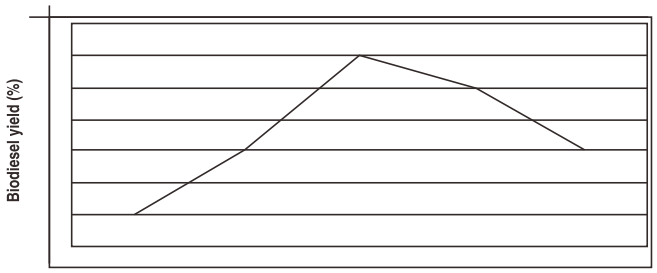

Fig. 2. Effect of methanol: oil (molar ratio) on biodiesel yield

Conclusion :

The present project work showed that biodiesel produced from the seed oil of *Madhuca indica* is an effective alternative source of energy as the parameters such as density and viscosity of biodiesel was almost similar to petrodiesel. It is a form of renewable source of energy and it has unique combustion quality as an oxygenated based fuel. Use of biodiesel as a conventional diesel will result in substantial reduction of unburnt hydrocarbon, carbon monoxide by approximately by 50% and carbon dioxide by 78.8% on a net life cycle because the carbon in biodiesel emission is recycled from carbon that is already present in atmosphere.

The use of *Madhuca indica* showed an effective result in biodiesel production as the free fatty acid of its oil was found to be upto 18% which was reduced to less than 2% after the process of transesterification. The biodiesel production was optimized with reaction temperature (45^0C), reaction time (8-10hours) and molar ratio of oil and methanol (1:6) which increased the yield of oil by 85% . Lipase catalysed biodiesel production also proved to be an effective way to form biodiesel for industrial application as well as for research purpose. On the basis of our result, it can be concluded that “Lipase catalysed biodiesel production by transesterification of oil extracted from

seeds of *Madhuca indica* "is an effective method for biodiesel production as it is an eco friendly and convenient source of biodiesel.

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