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Enzymatic Preparation of Biodiesel from Mahua Oil (*MadhucaIndica*) and its Characterization

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ABSTRACT

Alternative energy sources are supposed to be the most challenging job in the future world. Mahua oil has been utilized in the present research study for this purpose. The transesterification reaction between mahua oil and methanol has been optimized for the production of biodiesel in the presence of 8% biocatalyst, Novozyme 40013 at 6:1 molar ratio of oil to methanol at a reaction temperature of 65°C for 7 hrs of continuous stirring with 550 rpm of mixing intensity. The conversion achieved was 93.56%. The physicochemical properties of the prepared biodiesel is almost at par with the diesel fuel which ensures its use as fuel without modification of the engine. The study shows that mahua oil can be used as an important raw material for the production of alternative sources of energy.

Keywords: Biodiesel, mahua oil, Novozyme 40013, transesterification

1. INTRODUCTION

The depletion of non-renewable energy sources induces to think the academicians, researchers for alternative renewable energy sources in future. Biodiesel is one of the alternative energy sources and achieved a lot of attention during the last few decades. Different vegetable oils like mustard, soybean, groundnut, sunflower, rapeseed, sesame, rice bran etc are used as raw materials for the production of biodiesel but it puts a tremendous pressure on food and also on import of edible oils. So the need for non-edible sources for the production of biodiesel seems to be only option to meet the demand of alternative energy sources (1). For this purpose, non-edible vegetable oils like JatrophaCurcas(2-5), Karanja (*Pongamiapinnata*) (6, 7), Mahua*(MadhucaIndica)*(8, 9), Undi, Castor etc play an important role for biodiesel production and evaluated as diesel fuel substitute. Among the non-edible vegetable oil sources, mahua oil has been attended as one of the cost effective sources for this purpose (10).

The Mahua tree (Madhucaindica) (Fig. 1) belongs to the Sapotaceae family with a medium to large height found mostly in India (11). This tree also grows in some part of Central Africa. Madhucaindica and Madhucalongifolia are two major species found in India. The annual production of mahua oil in India reaches up to 60 million tonnes. Mahua tree isfound to be highly tolerant and thrives well in deep clay soil.

Figure 1. Mahua tree

Mahua tree can grow quickly with high adaptability to arid environments. So mahua oil is a potential oil for biodiesel production as alternative renweabl energy sources. Many researchers tried to produce biodiesel from this oil. Jeffrey et al (12) evaluated mahua oil for biodiesel production by two step transesterification reaction and identified performance and emission characteristics. Ghadge and Raheman (9) prepared biodiesel production from mahua oil with high free fatty acids. Panigrahi et al (13) analysedmahua biodiesel blends with diesel fuel in a C.I.engine and identified satisfactory results. Puha et al (14) identified that performance of mahua oil methyl ester is better compared to other esters on the basis of performance and emissions. Sukumar et al (15) studied the performance and emission characteristics of mahua ethyl ester in a four stroke direct injection diesel engine and identified good results. Pugazhvadivu and Sankaranarayanan (16) studied diesel engine using mahua oil as fuel and satisfactory results are obtained.

Ghadge and Raheman (17) optimized the process for biodiesel production from Mahua (Madhucaindica) oil using response surface methodology. Other researchers (18-24) also studied the mahua oil as a source of biodiesel and identified the blending compositions of mahua biodiesel and diesel fuel as an alternative fuel in future. Very little study have been made for the production of biodiesel from mahua oil using biocatalyst. Present authors have tried to optimize the reaction parameters for biodiesel from Mahua oil (MadhucaIndica) oil using non specific enzyme Novozyme 40013 (*Candida antarctica*) as catalyst. 93% conversion has been achieved and the properties of mahua methyl ester were compared with diesel standard which showed satisfactory results.

2. MATERIALS

Mahua oil was collected from Rishabh Oils Pvt Ltd, Kolkata, West Bengal. Novozyme 40013, an immobilized nonspecific lipase from *Candida antarctica* was used as catalyst in the reaction with ester synthesis activity of 10000 propyl laurate unit/g. Methanol was purchased from Scientific and Laboratory Instrument Co., Kolkata. Except otherwise specified all other chemicals were A.R. Grade.

3. METHODS

A. Transesterification of mahua oil

Initially 500 mL of mahua oil was filtered and taken in an Erlenmeyer flask and heated up to 80 °C to drive off moisture by continuous stirring for about 1 h. After that, methanol was added to it for transesterification reaction through stepwise manner in an appropriate proportion using solvent hexane at a specified temperature for 8 hours. Enzyme Novozyme 40013 was added in definite proportion (w/w) to the reaction mixture. The progress of reaction or production of biodiesel was monitored by thin layer chromatographic (TLC) method and the typical yield of each reaction product was determined separately by column chromatography using silicic acid as an adsorbent and 160 mL of hexane diethyl ether (99:1) as eluting solvent.

At the end of the reaction, the product was filtrated through separating funnel to remove the enzyme and allowed to separate. The lower layer was then evaporated under vacuum in order to remove excess methanol and the final product was collected. The enzyme was washed with hexane, dried and reused for the next experiment. Biodiesel characterization was done according to the American Standard Testing Method (ASTM). Values are reported as mean ± s.d., where n = 3 (n = no of observations).

B. Gas chromatographic analysis

Fatty acid composition of mahua oil was determined by a gas liquid chromatographic (GLC) method after converting into methyl ester. The HP 5890A GLC was connected with a HP 3390A data integrator. The GLC was fitted with a glass column (1.83 m X 3.175 mm id) packed with 10% DEGS supported on Chromosorb – WHP (100/200 mesh) of HP make. The oven temperature was programmed from 100 to 190 °C at 5° per min. The injector and detector block temperatures were maintained at 230 and 240°C, respectively. IOLAR-2 nitrogen was used as the carrier gas (flow rate 30 mL/min). The fatty acid esters peak was identified and calibrated with standard methyl esters. Data were represented an averages of three determinations.

4. RESULTS AND DISCUSSIONS

C. Analysis of mahua oil

The physicochemical properties and fatty acid profiles of mahua oil are shown in Table 1 and Table 2 respectively. It has been observed from Table 1 that mahua oil has higher flash point at nearly 228 °C with a kinematic viscosity of nearly 23.61 mm/s^2 at 40^oC. Higher iodine value (73.33) agrees with higher percentage of unsaturated part of this oil. It has been observed from Table 2 that the oil contains nearly 57% unsaturated fatty acids while saturated fatty acids part contributes nearly 41%. Apart from that, mahua oil contains negligible amounts of lauric acid and myristic acid.

Table 1: Physicochemical properties of mahua oil

Properties	Values	Test methods
Colour	Pale yellow	ASTMD-1981
Density at 15^{o}C(Kg/m3)	961±1.275	ASTMD 5002
Kinematic viscosity at 40^{o}C (mm2/s)	23.61±0.657	ASTM-D445
Iodine value	73.33±0.785	ASTMD 5554
Calorific value (MJ/Kg)	37.56±0.561	ASTMD-4868
Flash point (^{o}C)	228±1.101	ASTMD-93
Carbon residue (%)	4.1±0.011	ASTMD-524
Ash content (% w/w)	0.86±0.005	ASTMD 2584
Acid value, mg KOH	21.54±0.125	------

Table 2: Fatty acid composition of mahua oil

Fatty acid	Amount
Lauric acid (C12:0)	0.56±0.005
Myristic acid (C14:0)	0.71±0.006
Palmitic acid (C16:0)	19.65±0.034
Stearic acid (C18:0)	21.34±0.027
Oleic acid (C18:1)	43.18±0.075
Linoleic acid (C18:2)	13.78±0.031

D. Analysis of molar ratio of methanol and mahua oil

For the optimum production of biodiesel form mahua oil, different molar ratios of alcohol to mahua oil have been studied in the present research investigation. Among the molar ratios studied from 1:1 to 7:1 (MeOH: mahua oil), it has been observed that 6:1 molar ratio is the optimum ratio for the maximum conversion as shown in Fig. 2. Enhancing the ratio of alcohol to mahua oil does not increase the conversion as evidenced from Fig. 2. It may be due to the fact that increasing the amount of alcohol does not enhance the possible collision among reactants with the catalyst which is essential for higher conversion of reaction process.

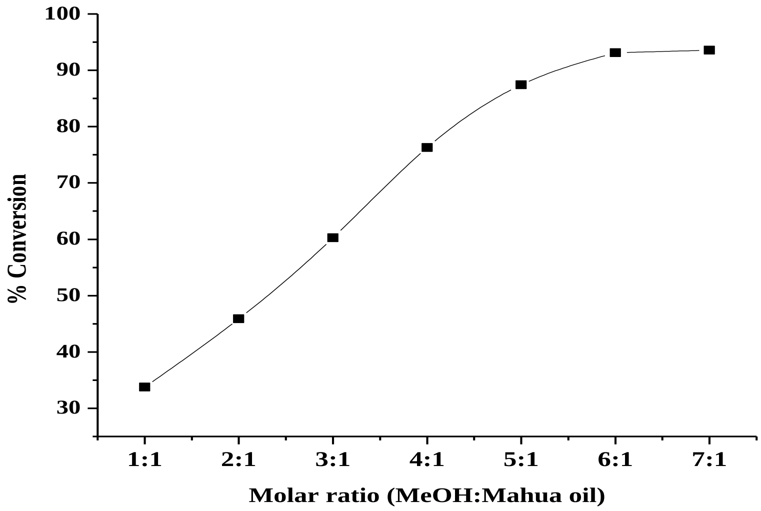

Figure 2. Analysis of molar ratio of alcohol to mahua oil for optimum conversion of biodiesel

E. Analysis of reaction temperature

Temperature plays an important rolefor the progress and completion of reaction. As transesterification reaction depends on activation energy of the reaction and the activation energy is proportional to applied temperature so temperature study identifies the optimum conversion of reaction. For the conversion of mahua oil to biodiesel with the reaction with methanol, a range of temperature from 35°C to 75°C have been studied using 8% biocatalyst for 7 hrs of continuous stirring with 550 rpm. The temperature study has been envisaged through Fig. 3 and it has been observed that 65°C is

the optimum temperature for biodiesel conversion from mahua oil. Enhancing the temperature does not increase the conversion rate as the enzyme shows its maximum efficiency at a certain temperature. Beyond that particular temperature, probably the deactivation of enzyme starts which has an effect on the rate of conversion of biodiesel.
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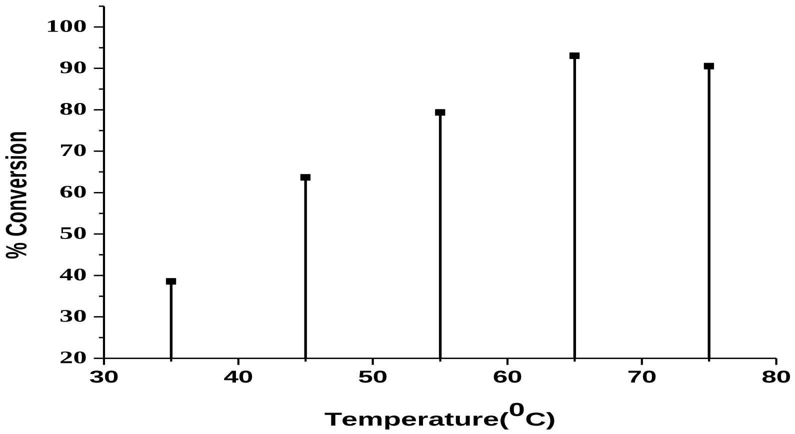

Figure 3. Analysis of reaction temperature for optimum conversion of biodiesel from mahua oil and methanol

F. Analysis of free fatty acids

During the transesterification reaction, free fatty acids of the reaction mixture decreases as the reaction proceeds. The study of the amount of free fatty acids during the reaction has been analysed which is shown in Fig. 4. It has been observed from the Fig. 4 that initially the rate of consumption of free fatty acids was high because at the start of the reaction the rate of reaction was higher. As the reaction proceeds, the rate of consumption of free fatty acids was slow and finally nearly 1% free fatty acids existed in the reaction mixture. Consumption of free fatty acids actually indicates the reaction towards completion.

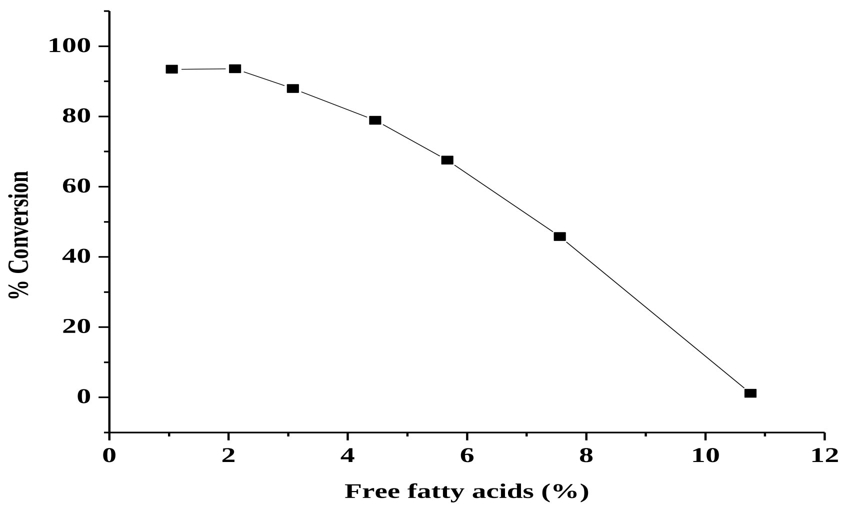

Figure 4. Analysis of free fatty acids during the transesterification reaction of mahua oil and methanol

During the progress of the reaction, biodiesel or methyl ester has been formed. But along with this, free fatty acids, triacylglycerols (TAGs), diacylglycerols (DAGs) and momoacylglycerols (MAGs) decrease as shown in Table 3. It has been observed from Table 3 that initially the rate of reaction is very fast in the presence of enzyme catalyst but as the reaction proceeds, the reaction rate would be slower. Table 3 shows the composition of mahua oil at the start of the reaction and after every two hours.

Table 3: Composition of reaction mixture at different time intervals

Materials	0 hr	2 hrs	4 hrs	6 hrs	8 hrs
TAG	77.51±0.784	51.34±0.087	34.58±0.102	13.78±0.027	1.34±0.005
DAG	6.21±0.023	4.56±0.011	2.48±0.002	1.87±0.007	1.23±0.007

MAG	3.78±0.012	2.22±0.034	1.67±0.003	0.97±0.001	0.56±0.005
FFA	10.56±0.034	5.37±0.031	3.02±0.007	2.07±0.012	1.07±0.001
Methyl ester	0.00	55.37±0.076	75.36±0.786	86.45±0.063	93.56±0.051

G. Characteristics of mahua biodiesel

Physicochemical properties of mahua biodiesel was compared with diesel fuel as shown in Table 4. It has been observed from Table 4 that the characteristics of biodiesel are comparable with biodiesel standards in most of the properties. Higher flash point of mahua biodiesel is significant and desirable also due to safe handling than diesel fuel. The calorific value of diesel fuel is higher than biodiesel but with regard to other characteristics, mahua biodiesel can be used safely without modification of engines.

Table 4: Comparative characteristics of mahua biodiesel

Properties	Diesel fuel	Mahua biodiesel	ASTM D6751
Density at 15^{o}C(Kg/m3)	841	869±0.788	860-900
Calorific value (MJ/Kg)	45	38±0.023	-----
Kinematic viscosity at 40oC (mm2/s)	3.17	3.9±0.011	1.9-6.0
Flash point (^{o}C)	63	211±0.627	Min 130
Carbon residue (%)	0.1	0.2	-----
Cetane number	51	53±0.312	------
Fire point (^{o}C)	101	212±0.444	Min 145
Ash content (%)	0.01	0.02	<0.02

CONCLUSION

Mahua oil has been used for the production of biodiesel which is low cost and easily available raw material. Process optimization has been done for this purpose. Enzyme Novozyme 40013 is used as catalyst for the transesterification reaction which ensures to avoid rigorous reaction conditions. The enzyme can be recycled further many times for the production of biodiesel or in any other reaction. The use of mahua oil for the production of alternative sources may be a good source for the mitigation of non-renewable energy sources in future.

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