Research Article

Biodiesel from Karanja (*Pongamia Pinnata* L.) Oil Using Non Specific Enzyme *Candida Antarctica*

Sumit Nandi* and Rupa Bhattacharyya

Department of Chemistry, Narula Institute of Technology, Maulana Abul Kalam Azad University of Technology, Kolkata, West Bengal, India

Abstract

The present work deals with the critical analysis and optimization of process parameters for the enzymatic preparation of biodiesel from Karanja (*Pongamia pinnata L.*) oil and methanol through transesterification reaction. This oil is one of the suitable and cost effective sources for this purpose. Reaction controllers like molar ratio of oil and alcohol, temperature, stirring speed, reaction time, concentration of catalyst have been analysed and experiments showed that using 5% non specific enzyme Novozyme 435 (*Candida antarctica*) as catalyst with 6:1 molar ratio of methanol to Karanja oil, 600 rpm of mixing intensity, 65±2^{0}C temperature are the optimum condition for 6 hrs duration of reaction. The fuel properties of Karanja methyl ester or biodiesel like density, kinematic viscosity, flash point, pour point, calorific value, cloud point etc. are compared with diesel fuel according to the ASTM standards which suggest the use of Karanja methyl ester or biodiesel in the engine without modification.

Keywords: Biodiesel, *Candia antarctica*, *Pongamia pinnata L.*, Karanja oil

***Correspondence**
Author: Sumit Nandi
Email: sumitnandi5@gmail.com

Introduction

The scarcity of non renewable energy sources and increasing environmental degradation caused by fossil fuels, biodiesel (BD) is gaining importance throughout the world for the last few decades as alternative source of energy due to its renewable, nontoxic, eco friendly and biodegradable nature. The use of edible oil resources like mustard, soybean, groundnut, sunflower, rapeseed etc. for the production of BD put a tremendous pressure on food and also on import of edible oils. So the need for non edible sources for production of BD seems to be only option for cultivation and extraction to meet the demand of alternative energy sources [1]. In this situation, non edible oils obtained from Jatropha Curcas [2-5], Karanja (*Pongamia pinnata*) [6, 7], Mohua [8, 9], Undi, Castor etc are evaluated as diesel fuel extender. Amongst them, Karanja oil is one of the cost effective and suitable sources for this purpose. Karanja tree is a wonderful tree which is medium sized and found almost throughout India. The common name of the oil is Karanja seed oil and the botanical name Pongamia glabra of Leguminaceae family. Karanja tree is said to be highly tolerant to salinity and can be grown in different soil textures like stony, sandy and clayey. It can grow in humid as well as subtropical environments. So Karanja oil can be utilized as a suitable feedstock for BD production in country like us [10-12].

Production of BD from non edible oil has been studied by several researchers in the presence of chemical as well as biological catalyst. In our earlier work, analysis of BD production from Jatropha Curcas oil has been done and good quality BD has been obtained using non specific enzyme Novozyme 435 [2, 3]. BD production from Karanja oil has been studied by many researchers through chemical catalytic method [11, 13, 14]. But very limited study has been made so far for enzymatic production of BD through transesterification reaction between Karanja oil and methanol. Present authors have tried to optimize the reaction parameters for BD from Karanja (*Pongamia pinnata L.*) oil using non specific enzyme Novozyme 435 (*Candida antarctica*) as catalyst. 92% conversion has been achieved and the properties of Karanja methyl ester or BD were compared with diesel standard which showed satisfactory results.

Experimental

The Karanja oil used in this study was provided by M/s. A.S. Oil Mills, Burdwan, West Bengal, India. The enzyme used in the following studies was Novozyme 435 (*Candida antarctica*) immobilized lipase which was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India. The chemicals used in this work methanol and hexane were purchased from S.D. Fine Chemicals (Mumbai, India). Except otherwise specified all other chemicals used were A.R. Grade.

For transesterification reaction of Karanja oil and alcohol in the presence of enzyme, initially 250 mL of crude oil was taken in an Erlenmeyer flask and heated up to 80^0C to drive off moisture by continuous stirring for about 1 h. After that, transesterification reaction was carried out by stepwise addition of alcohol in an appropriate proportion using solvent hexane fitted with a water condenser and stirred by a magnetic stirrer at a specified temperature for 6 hours maintaining other reaction conditions. To the reaction mixture, immobilized enzyme Novozyme 435 was added in definite proportion (w/w). Stepwise addition of methanol was allowed to minimize the deactivation of enzyme.

During the reaction, continuous sampling and analysis were done by withdrawing the sample in to a capped vial and removing enzyme through centrifugation. 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. TLC was done by spotting the lipid mixture on a silica-gel G plate (0.2 mm thick) using hexane-diethyl ether-acetic acid (90:10:1) as a developing solvent [15].The lipid spots were identified by iodine absorption with triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG) and BD as standard. The composition of different karanja esters using different alcohols was determined by column chromatography using silicic acid as an adsorbent and 160 mL of hexane-diethyl ether: 99:1 as eluting solvent [16]. After completion of reaction, 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).

Results and Discussions

Good quality as well as optimum conversion of BD depends on the initial characteristics of the crude oil. **Table 1** shows the fatty acid composition and characteristics of crude Karanja oil which was used for the preparation of BD. It has been observed from Table 1 that it contains mainly palmitic, stearic, oleic and linoleic acid with maximum percentage of oleic acid. Apart from that the crude oil contains Eicosanoic acid, Docosanoic acid and Tetracosanoic acid. Its properties were established to ascertain the suitability for BD production using enzyme catalyst and to compare with diesel fuel.

For identifying optimum reaction parameters, transesterification reaction between Karanja oil and methanol was carried out by varying a specific parameter maintaining other parameters constant. So for determining optimum molar ratio of alcohol to oil, the reaction was carried out at 65 ± 2^0C using 5% (wt/wt) immobilized enzyme for 6 hours at 600 rpm by varying molar ratios as shown in **Figure 1**.

Table 1 Fatty acid composition and characteristics of crude Karanja oil

Fatty acid	% (w/w)	Characteristics	Values
Palmitic acid	11.36±0.101	Acid value (mg KOH/gm)	3.23 ±0.003
Stearic acid	7.26±0.081	Flash point (^{0}C)	221±1.23
Oleic acid	52.28±0.335	Cloud point (^{0}C)	3.5±0.004
Linoleic acid	15.11±0.119	Pour point (^{0}C)	-4±0.023
Eicosanoic acid	1.21±0.006	Density (gm/cc)	0.933±0.105
Docosanoic acid	4.73±0.015	Kinematic Viscosity (mm2/s) (40^0C)	39.23±0.803
Tetracosanoic acid	1.03±0.001	Iodine value	87±1.21
		Unsaponifiable matter	2.9±0.012
		Carbon residue (w/w%)	1.54±0.001
		Specific gravity	0.937±0.008
		Calorific value (Kcal/Kg)	8742±2.67
		Cetane number	42±0.95

It has been observed from the figure that at 6:1 molar ratio of methanol to Karanja oil, maximum biodiesel is obtained. Further enhancing of molar ratio did not increase the production as evidenced from the figure. Similarly, by varying other reaction parameter temperature from 25 to 75^0C, it has been observed from **Figure 2** that maximum biodiesel is obtained at 65^0C and no further increment of biodiesel conversion is achieved by increasing temperature beyond that. This may be due to the fact that maximum enzyme activity has been achieved at a certain temperature beyond which it has been deactivated. Higher temperature also volatilize methanol which prevents maintaining the proper ratio of methanol: oil.

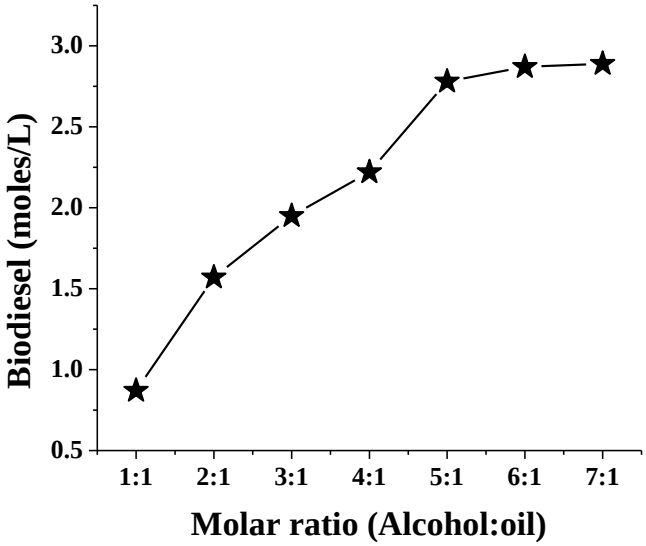

Figure 1 Effect of molar ratio

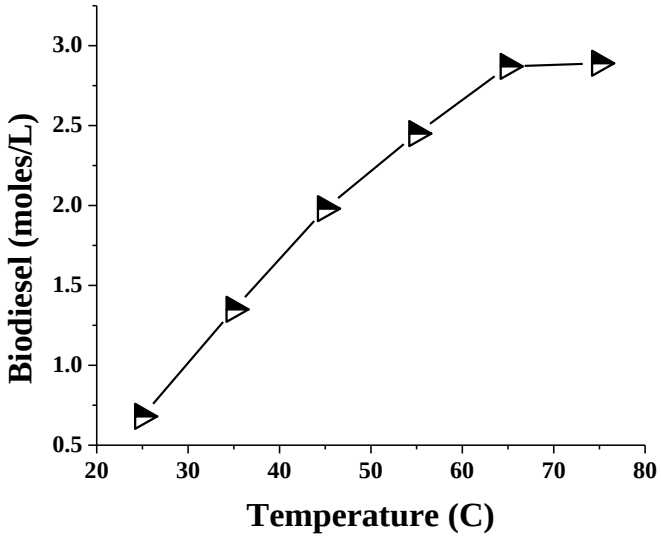

Figure 2 Effect of temperature

Concentration of catalyst plays a significant role for the maximum conversion of biodiesel. For the determination of proper enzyme concentration, transesterification reaction was investigated at 6:1 molar ratio of alcohol to oil at 65 ± 2^0C for 6 hours by varying catalyst concentration from 2-6%. **Figure 3** shows the effect of changing the concentration of enzyme on the productivity of biodiesel. It indicates that increasing concentration of enzyme enhances BD production up to 5% enzyme concentration but after that no significant increase in biodiesel production was observed by further increasing the enzyme concentration. This may be due to the fact that higher amount of enzyme contributes higher amount of active sites but all active sites cannot be exposed to the substrates due to enzyme aggregation.

Similarly, by changing the mixing intensity from 200 to 800 rpm maintaining other reaction parameters unchanged, it has been found from **Figure 4** that 600 rpm is the optimum stirrer speed which contributes maximum production.

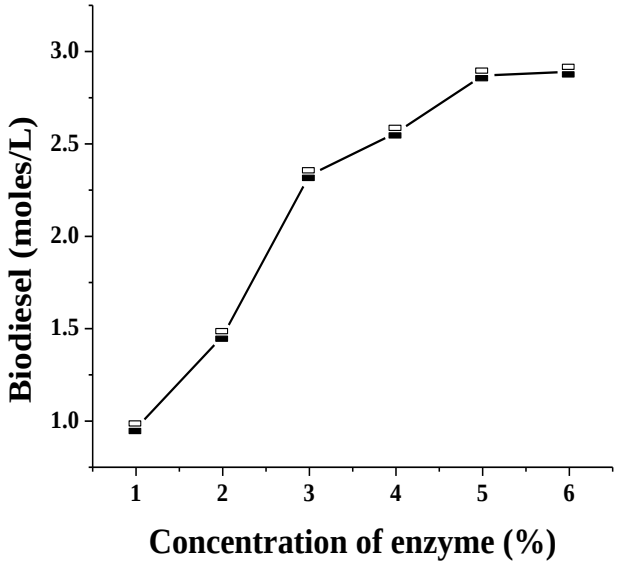

Figure 3 Effect of enzyme concentration

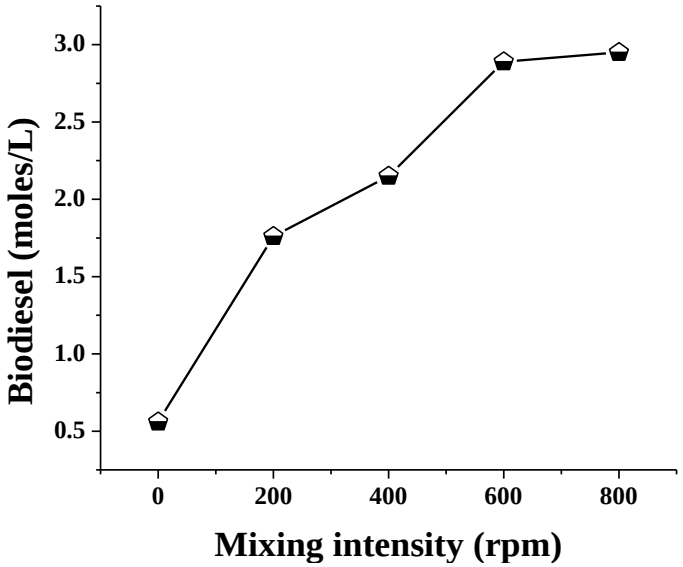

Figure 4 Effect of mixing intensity

Biodiesel characterisation

The performance and emission characteristics of the diesel engine can only be obtained by using good quality of BD. So BD from Karanja oil and methanol in our study has been analyzed and compared with the biodiesel standards. **Table 2** shows that the characteristics of BD produced in our study are quite comparable with the BD standards. It has been observed from Table 2 that though calorific value of Karanja BD is somewhat less than conventional diesel fuel but with regard to other characteristics Karanja BD is analogous to diesel fuel.

Table 2 Characteristics of Karanja BD and diesel fuel

Characteristics	Karanja BD	Diesel fuel
Density (gm/cc)	0.858±0.001	0.840
Flash point (^{0}C)	215±0.881	56
Fire point (^{0}C)	222±0.778	62
KinematicViscosity@ 40^{0}C (Cst)	4.82±0.047	3.02
Specific gravity	0.879±0.001	0.85
Calorific value (Kcal/Kg)	3650±2.567	4285
Acid value (mg/KOH)	0.42±0.001	0.36
Moisture (%)	0.02	0.02
Cetane number	41±0.234	49

Conclusion

Feasibility study for biodiesel production from crude Karanja oil (*Pongamia pinnata L.*) has been successfully carried out in our present research investigation. The reaction parameters are optimized which contributes nearly 92% conversion of biodiesel from crude Karanja oil. The catalyst used in our study is non specific enzyme Novozyme 435 (*Candida antarctica*) in the presence of solvent hexane. The enzyme catalyst may be recycled which is our future plan of work and recycling process helps to minimize the cost of production. Moreover, the properties of Karanja methyl ester (biodiesel) show similarity with diesel fuel which is also encouraging. Our research investigation shows the potential use of non edible oils as alternative source of energy which may be useful in the present depleting condition of non renewable energy sources.

References

[1] B. K. Banwal and M. P. Sharma, Renewable and Sustainable Energy Reviews, 2005, 9(4), 363-378.
[2] S. Nandi and R. Bhattacharyya, Research Journal of recent Sciences, 2015, 4(ISC-2014), 44-50.
[3] S. Nandi and S. Ganguly, Journal of Chemical, Biological and Physical Sciences, 2016, 6(4), 1419–1428.
[4] M. S. A. Ginting, M. T. Azizan and S. Yusup, 2012, Fuel, 93, 82-85.
[5] P. K. Roy, S. Datta, S. Nandi and F. A. Basir, Fuel, 2014, 134, 39-44.
[6] Y. C. Sharma and B. Singh, Journal of Fuel, 2008, 87, 1740-1742.
[7] S. Karmee and A. Chadha, Journal of Bioresource Technol, 2005, 96, 1425-1429.
[8] S. K. Padhi and R. K. Singh, Journal of Chemical and Pharmaceutical Research, 2010, 2(5), 599-608.
[9] S. V. Ghadge and H. Raheman, Biomass and Bioenergy, 2005, 28, 601-605.
[10] G. Dwivedi, S. Jain and M. P. Sharma, Smart Grid and Renewable Energy, 2011, 2,184-189.
[11] S. N. Bobade and V. B. Khyade, Research Journal of Chemical Sciences, 2012, 2(8), 43-50.
[12] L. C. Meher, S. N. Naik and L. M. Das, Journal of Scientific and Industrial Research, 2004, 63, 913-918.
[13] Vivek and A. K. Gupta, Journal of Scientific and Industrial Research, 2004, 63, 39-47.
[14] S. Gangil, C. Mewar, R. K. Singh and B. Modhera, Biofuels, 2015, 6, 377-381.
[15] M. Kates, Techniques of lipidology, Separation of Lipid Mixtures, North-Holland Publishing Company, Amsterdam, London, 1972a, 428-435.
[16] M. Kates, Techniques of lipidology, Separation of Lipid Mixtures, North-Holland Publishing Company, Amsterdam, London, 1972b, 397-405.

Publication History
Received 05th Jan 2018
Revised 28th Jan 2018
Accepted 05th Feb 2018
Online 28th Feb 2018