

SUSTAINABLE BIODIESEL PRODUCTION: ENZYMATIC CONVERSION OF *MORINGA OLEIFERA*

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ABSTRACT: This study investigates the sustainable production of biodiesel from mature *Moringa oleifera* seeds, a non-edible and underutilized biomass that does not compete with food resources, thereby supporting food-energy balance and circular bioeconomy goals. Biodiesel was produced via transesterification using *Candida antarctica* lipase immobilized on functionalized activated carbon (FAC) as a green biocatalyst. Moringa oil was extracted through Soxhlet extraction using hexane, and its physico-chemical properties including acid value, iodine value, density, moisture content, viscosity, and saponification value were characterized to assess its suitability as a sustainable biodiesel feedstock. To enhance enzymatic efficiency and reusability, *Candida antarctica* lipase was purified and immobilized on FAC treated with various acids (HCl, HNO₃, H₂SO₄), with hydrochloric acid-functionalized FAC (HCl-FAC) showing the highest immobilization capacity (6.022 U/g). Immobilization conditions (time, temperature, pH, agitation) were optimized using One-Factor-at-a-Time (OFAT) screening and Face Centered Composite Design (FCCCD) under Response Surface Methodology (RSM), yielding optimal conditions of 40°C, pH 6, and 24 hours. Further optimization of biodiesel synthesis was performed, considering methanol-to-oil ratio, temperature, catalyst concentration, and reaction time. Maximum biodiesel yield (94.01%) was achieved under optimal sustainable conditions: methanol-to-oil ratio of 4:1, 40°C, 4% catalyst loading, and 24 hours reaction time. The kinetic analysis indicated a pseudo-first order reaction with an activation energy of 43.126 kJ/mol and a frequency factor of $1.758 \times 10^8 \text{ min}^{-1}$. Post-reaction, the biodiesel was characterized by its iodine value, acid composition, kinematic viscosity, density, cloud point, and pour point. The fuel properties conformed to ASTM D6751 and EN 14214 standards, confirming their high quality and environmental compatibility. Overall, this study demonstrates a sustainable, efficient, and eco-friendly approach to biodiesel production from *Moringa oleifera*, reinforcing its potential role in renewable energy strategies and low-carbon fuel alternatives.

KEYWORDS: *Sustainability, Biodiesel, Optimization, Moringa Oleifera, Enzymatic conversion*

1. INTRODUCTION

Recent studies highlight that enzymatic conversion of *Moringa oleifera* oil into biodiesel is gaining traction due to its eco-friendly process, high yield potential, and reduced need for harsh chemical catalysts. Research between 2022–2025 emphasizes optimization of reaction parameters, novel biocatalysts, and blending strategies to meet ASTM fuel standards [1,2].

Recent advances in enzyme immobilization for biodiesel production have focused on improving operational stability and reusability. A 2026 comprehensive review in Next Energy

consolidates advances involving lipases, phospholipases, and multi-enzyme systems [3]. The increasing demand for sustainable and renewable energy sources has intensified research into biodiesel as an alternative to fossil diesel. Biodiesel is biodegradable, non-toxic, and can be produced from renewable lipid feedstocks through transesterification reactions [4].

In response to the limitations associated with homogeneous chemical catalysts, enzymatic biodiesel production employing lipases has attracted considerable attention owing to its environmentally benign nature and its capacity to utilize low-grade oils. Among the various non-edible feedstocks, *Moringa oleifera* oil has emerged as a promising candidate due to its advantageous physicochemical characteristics and abundant availability across tropical and subtropical regions [5].

In response, the European Union set renewable energy targets for 2020 to reduce fossil fuel dependence, emphasizing that human development should not come at the expense of environmental degradation. Many developing countries have adopted similar strategies, aiming to curb the release of harmful pollutants by transitioning to renewable energy [6]. The production of biodiesel from non-edible vegetable oils has gained significant traction as a sustainable alternative to first-generation biodiesels. *Moringa oleifera*, a drought-resistant tree native to South Asia and widely cultivated in tropical regions like Malaysia, has emerged as a prime candidate due to its high seed oil content (typically 35–45%) and favorable fatty acid profile [7].

Fossil fuel depletion and environmental concerns have accelerated the search for renewable biofuels. Biodiesel, a promising substitute for petroleum diesel, offers biodegradability, renewability, low sulfur, and lower emissions. It is commonly produced via transesterification of vegetable oils, animal fats, or waste oils with methanol or ethanol. Recently, lipase-catalyzed enzymatic transesterification has gained attention as a greener alternative to chemical catalysts [8]. While industrial-scale renewable sources such as hydroelectric, solar, wind, and biofuels are increasingly used, they are not yet economically viable to fully match the energy output of fossil fuels [9].

Current research trends have focused on overcoming the principal challenges of enzymatic biodiesel production, namely high enzyme cost and reduced enzyme activity caused by methanol inhibition. Researchers have investigated stepwise alcohol, enzyme immobilization, and alternative reactor designs. Microreactors, ultrasonic-assisted systems, and membrane reactors have shown potential for improving mass transfer and reducing reaction times. Process intensification technologies have demonstrated improvements in biodiesel yield and enzyme reusability [10].

2. COMPOSITION OF BIODIESEL

The fatty acid composition of biodiesel varies depending on the type of feedstock oil used in its production. Typically, the most common straight-chain fatty acids in biodiesel contain between 16 and 18 carbon atoms. However, this composition can differ significantly, particularly with tropical oils, which often have shorter-chain fatty acids. During transesterification, minor constituents such as mono- and diacylglycerols, residual triacylglycerols, methanol, sterols from vegetable oils, and sterol glucosides are also formed [11]. Triglycerides the primary component of feedstock oils contain three types of fatty acids: saturated, monounsaturated, and polyunsaturated, the latter having two or more double bonds. While various feedstock oils are available for biodiesel production, the selection should be based on their potential for high yield and efficient conversion, as the fatty acid profile directly influences biodiesel quality. Ideally, feedstocks rich in monounsaturated fatty acids are

preferred. In contrast, oils high in polyunsaturated fatty acids, such as sunflower oil, often produce biodiesel with lower oxidative stability and poor cold flow properties as in figure 1 [12].

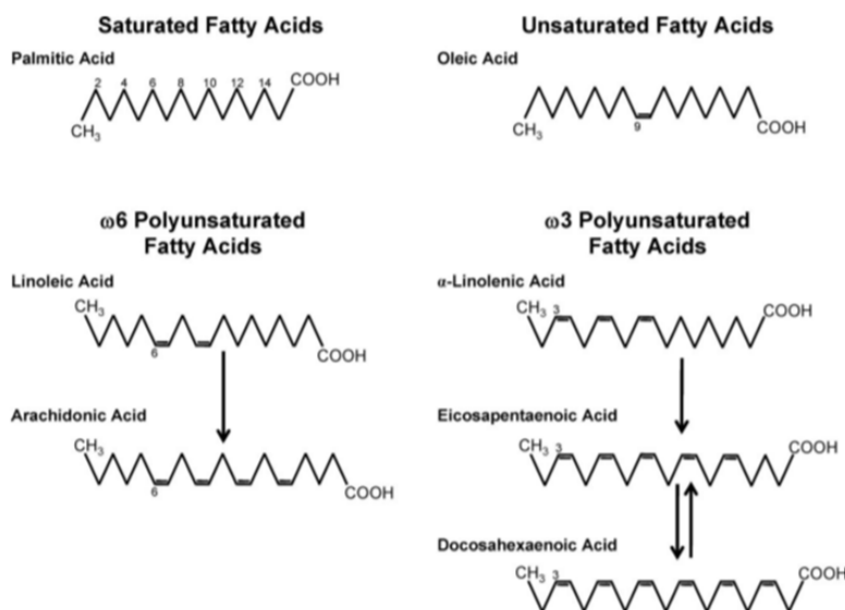


Fig. 1. Types of fatty acids - saturated, unsaturated, polyunsaturated [13]

To ensure consistent engine performance and prevent issues such as injector coking, the quality of industrial-scale biodiesel production has been standardized globally [14]. Biodiesel, whether derived from edible or non-edible feedstocks, must meet the specifications set by regulatory bodies such as the American Society for Testing and Materials (ASTM) and the European Committee for Standardization (EN). These organizations define strict guidelines to ensure the safety and compatibility of biodiesel for engine use. Specifically, biodiesel must comply with ASTM D6751 in the United States and EN 14214 in Europe [15].

Table 1: Some standards for biodiesel according to ASTM and EN

Properties	ASTM D6751	EN 14214
Viscosity	1.9-6.0 mm ² /s	3.5-5.0mm ² /s
Cloud point	Not below 6°C	-
Pour Point	Not below 6°C	-
Acid value	0.80 mgKOH/g maximum	0.5 mgKOH/g maximum
Density	-	860-900kg/m ³
Iodine value	-	120 maximum
Water content	-	24 mg/kg maximum

Table 2 presents the fuel composition of various fatty acid methyl esters (FAMES) compared to the alkane components of petro-diesel. The cetane number of FAMES depends on the fatty acid chain length and degree of unsaturation. High levels of polyunsaturated esters can reduce oxidative stability, while excessive saturated esters can impair cold flow properties.

For biodiesel use in low-temperature environments, the cloud point is a critical parameter, indicating the temperature at which wax crystals begin to form helping to predict issues such as fuel filter plugging. Similarly, the pour point defines the lowest temperature at which the fuel can still flow freely, making it another key factor in evaluating cold weather performance.

Table 2: Comparison of properties of petro-diesel and biodiesel [11]

	Compound	Cetane Number	Kinematic Viscosity	Melting Point
Petrodiesel	Decane		0.97	-29.6
	Undecane		1.20	-25.5
	Tetradecane		2.09	5.82
	Hexadecane	100	2.93	18.12
	Heptadecane			22.0
	Octadecane			28.2
Biodiesel	Methyl decanoate	51.6	1.71	-13.48
	Methyl linoleate	38.2	3.65	-43.09
	Methyl stearate	101	5.85	37.66
	Methyl palmitate	85.9	4.38	28.48
	Methyl laurate	66.7	2.43	4.30

Biodiesel generally exhibits a higher boiling point than petro-diesel, primarily due to the more linear molecular structures and higher molecular weights of its components, such as methyl palmitate and methyl decanoate. As a result of these properties, biodiesel also has a higher flash point, the minimum temperature at which the fuel produces enough vapor to ignite making it safer to handle compared to petro-diesel.

2.1. *Moringa Oleifera*

Moringa oleifera is a widely cultivated plant recognized for its medicinal and nutritional properties, and it thrives in a variety of climates across the globe. In regions of South Asia and Africa, nearly all parts of the plant are traditionally used for healing purposes [15]. One of its notable advantages is its ability to grow on marginal land unsuitable for conventional food crops, making it a promising candidate for large-scale cultivation without compromising food security [16]. The leaves are rich in protein and antioxidants and are commonly used to combat malnutrition, particularly among infants and adults in India [17]. *Moringa oleifera* can yield up to 3 tons of seeds per hectare annually, with seeds containing approximately 40% oil by weight [18]. Importantly, the mature seeds are not a staple food source unlike the leaves, roots, or bark making them suitable for non-food applications such as biodiesel production [19].

The fatty acid content of *Moringa oleifera* oil was found to be below 1% in a study by [20], indicating that a single-step transesterification process is sufficient for its conversion into biodiesel as in Table 3. This eliminates the need for pre-treatment commonly required for other non-edible oils with high free fatty acid levels, thereby reducing overall production costs. Furthermore, biodiesel derived from moringa oil has a remarkably high cetane number of 67 among the highest reported for any biodiesel feedstock indicating superior combustion quality and cleaner burning compared to many other biofuels [21].

Table 3: Fatty acid composition of *Moringa oleifera* oil

Fatty acid	Percentage
Palmitic (16:0)	7.0
Palmitoleic (16:1)	2.0
Stearic (18:0)	4.0
Oleic (18:1)	78.0
Linoleic (18:2)	1.0
Linolenic (18:3)	-
Arachidic (20:0)	4.0
Behenic (22:0)	4.0

2.1.1. Research Gap

Current biodiesel production methods face significant challenges related to feedstock selection and environmental impact. The reliance on edible oils as primary feedstocks creates competition with food supplies, raising ethical and economic concerns. Moreover, conventional transesterification processes often lead to environmental degradation due to the release of pollutants and require costly post-treatment. The use of low-cost, non-edible oils is often limited by high free fatty acid (FFA) content, which promotes soap formation and reduces yield when using alkaline catalysts. Additionally, most catalytic processes involve harsh chemicals that are difficult to separate from the final product and are not environmentally sustainable. There is a lack of optimized, scalable biodiesel production systems that use non-edible, sustainable feedstocks like *Moringa oleifera* seed oil in combination with green, reusable biocatalysts. Specifically, research on the immobilisation of lipase enzymes onto activated carbon as a stable, efficient, and eco-friendly catalyst system remains limited. This represents a critical gap in developing a cost-effective, environmentally benign biodiesel production process suitable for long-term use particularly in biodiesel-importing nations like Malaysia seeking locally sourced, renewable energy alternatives.

3. METHODOLOGY

Biodiesel production from *Moringa oleifera* oil was conducted at the laboratory scale, with the transesterification process optimized by evaluating the effects of reaction time, temperature, agitation speed, catalyst loading, and methanol-to-oil ratio on the final biodiesel yield. The kinetics of free fatty acid (FFA) conversion into fatty acid methyl esters (FAME) were also investigated. The resulting biodiesel was subsequently characterized to assess its compliance with international quality standards, specifically EN 14214 and ASTM D6751.

3.1. Research Flowchart

The feedstock oil sample was characterized using multiple analytical methods recommended by the Association of Official Analytical Chemists (AOAC) and the Malaysian Palm Oil Board (MPOB). The catalyst employed in this study a lipase enzyme was derived from *Candida antarctica* under optimal growth conditions. The produced lipase was subsequently immobilized on acid functionalized activated carbon. Three acids hydrochloric acid, sulfuric acid, and nitric acid were evaluated as potential agents for enhancing the adsorption of lipase onto activated carbon. The effects of various parameters on both immobilization and transesterification processes were investigated and optimized using a Face-Centered Central Composite Design (FCCCD) within Design-Expert Software version 6.0.8. Furthermore, the kinetics of the transesterification reaction were analyzed to estimate the activation energy and frequency factor based on the Arrhenius equation. Finally, the biodiesel

produced was characterized to assess its compliance with international standards, specifically ASTM D6751 and EN 14214.

4. RESULTS AND DISCUSSION

The immobilization of *Candida antarctica* lipase on functionalized activated carbon was carried out in 250 mL shake flasks to evaluate the influence of reaction conditions. Analysis of individual parameter effects revealed that the maximum permissible amount of lipase was adsorbed onto the support matrix. Among the tested variables, an incubation time of 24 hours, temperature of 40 °C, and pH of 6 were identified as the optimal conditions for the hydrolysis process. These parameters were subsequently applied for the optimization of the immobilization procedure.

4.1 Experimental Design of Immobilisation

A Face-Centered Central Composite Design (FCCCD) using Design-Expert software (version 6.0.8) was employed to evaluate the interaction of parameters for optimal lipase immobilization. Regression analysis was applied to estimate the relationships between variables and develop a predictive model based on these interactions as seen in table 4.

Table 4: Centre points for influencing parameters for immobilisation of lipase on FAC

Parameter	-1	0	1
Time (hours)	12	24	36
Temperature (°C)	30	40	50
pH	5	6	7

Each experimental run varied time, temperature, and pH around the central points identified from the earlier One-Factor-at-a-Time (OFAT) analysis. The immobilization response ranged from 62.94% to 97.42%, as presented in Table 5. The highest responses were observed at the center point runs (Runs 1, 2, and 17). Statistical analysis indicated significant linear and quadratic effects of the parameters, while their interactions were found to be insignificant. Regression modelling was then performed to predict the response accurately and identify the variables exerting the greatest influence on immobilization efficiency.

Table 5: Experimental matrix for the immobilisation of lipase on functionalised activated carbon using FCCCD of Design Expert software

Run	Factors			Response	
	Time (hours)	Temperature (°C)	pH	Immobilisation experimental (%)	Immobilisation theoretical (%)
1	24	40	6	97.42	94.97
2	24	40	6	95.5	94.97
3	36	30	5	84.22	83.74
4	24	50	6	82.16	85.44
5	36	50	5	72.05	74.64
6	12	50	7	68.96	71.90
7	24	40	7	83.28	87.49
8	24	40	5	80.29	83.64
9	36	50	7	76.08	77.85
10	24	30	6	88.14	92.65

11	12	30	5	71.04	72.03
12	36	40	6	90.12	95.96
13	36	30	7	85.04	85.36
14	12	30	7	77.05	77.02
15	12	40	6	85.12	87.08
16	12	50	5	62.94	65.40
17	24	40	6	95.57	95.12

4.2 Experimental Design of Transesterification

A Face-Centered Central Composite Design (FCCCD) using Design-Expert software was employed to investigate the interaction of parameters for optimizing biodiesel yield. Each experimental run involved varying time, temperature, and catalyst loading around the central points established from the previous OFAT analysis as in table 6.

Table 6: Centre points influencing parameters for immobilisation of lipase on FAC

Parameter	-1	0	1
Time (hours)	12	24	36
Temperature (°C)	30	40	50
Catalyst loading (%)	2	4	6
Methanol:oil	2:1	4:1	6:1

The biodiesel yield ranged from 61.5% to 95.5%, as presented in Table 7. The highest yields were observed at the center point runs (Runs 6, 12, and 16). Statistical analysis revealed significant linear and quadratic effects for the parameters, while their interactions were determined to be insignificant.

Table 7: Experimental matrix for the production of biodiesel via transesterification using FCCCD of Design Expert software

Run	Factors				Response
	Time (hours)	Temp. (°C)	Cat. load (%)	Methanol: oil	Yield (%) actual
1	24	30	4	4:1	87.47
2	36	30	6	2:1	78.05
3	12	30	2	6:1	74.34
4	24	40	6	4:1	86.06
5	24	40	4	6:1	90.96
6	24	40	4	4:1	95.52
7	36	50	6	6:1	68.35
8	36	50	6	2:1	67.19
9	24	50	4	4:1	82.38
10	36	30	6	6:1	79.14
11	12	50	2	2:1	61.48
12	24	40	4	4:1	95.49
13	12	40	4	4:1	84.54
14	36	40	4	4:1	93.14
15	12	50	6	2:1	60.64
16	24	40	4	4:1	95.47
17	36	30	2	2:1	79.59
18	36	50	2	2:1	68.04

19	24	40	4	2:1	91.06
20	12	50	6	6:1	65.39
21	36	50	2	6:1	77.78
22	12	50	2	6:1	64.29
23	24	40	2	4:1	85.94
24	12	30	6	6:1	75.86
25	36	30	2	6:1	81.52
26	12	30	2	2:1	72.64
27	12	30	6	2:1	76.82

The 3D response surface plots shown in Figure 2 illustrate the interactions among the process parameters and their effects on the biodiesel yield from feedstock oil. While each parameter individually influenced the response, most parameter interactions were negligible, except for the interaction between reaction time and the methanol-to-oil ratio. The plots indicate that as reaction time increased, the effect of the methanol-to-oil ratio on biodiesel yield became more pronounced. This observation aligns with previous studies showing that methanol concentration significantly impacts biodiesel production; however, prolonged exposure to methanol can lead to lipase inactivation, reducing overall yield.

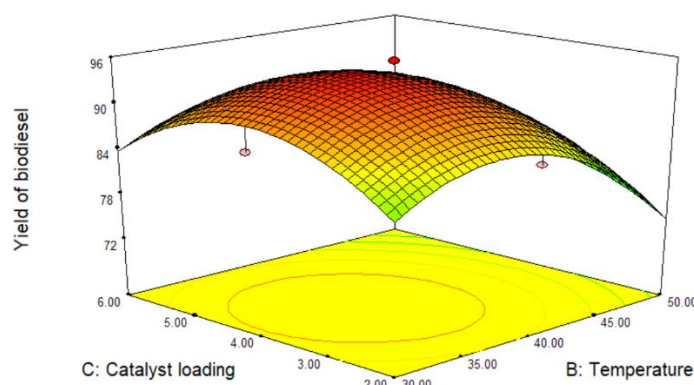


Fig. 2. 3D contour plots showing the Interaction between catalyst loading and temperature

The optimized conditions identified were temperature of 40 °C, catalyst loading of 4%, methanol-to-oil ratio of 4:1, and reaction time of 24 hours. These trends remained consistent across all contour plots, regardless of variations in other parameters. A notable decline in productivity was observed at temperatures above 40 °C, likely due to thermal inactivation of the lipase, like trends observed during immobilization optimization.

A control experiment conducted under the optimized conditions revealed that using a non-immobilized catalyst (free enzyme) resulted in a maximum biodiesel yield of 78.45%, indicating that immobilization significantly enhances biodiesel production.

4.3 Sustainable Biodiesel Characterisation

The properties of biodiesel are influenced by the origin of the feedstock oil, which is primarily determined by its fatty acid composition. During esterification and transesterification, physical and chemical transformations occur, sometimes leading to the formation of undesirable by-products [15]. Among these are polymers that, upon thermal degradation, produce monomeric and dimeric fatty acid esters. The presence of such compounds alters the molecular weight and volatility of the biodiesel, potentially reducing its

suitability for conventional engines due to increased internal fluid resistance and diminished combustion efficiency. In this study, the fuel properties of the produced biodiesel were evaluated against ASTM and EN specifications for industrial application as in table 8.

Table 8: Comparison of *Moringa* biodiesel produced with MPOB, ASTM and EN standards.

Fuel Properties	International biodiesel standards			<i>Moringa</i> biodiesel
	MPOB	ASTM D6751	EN 14214	
Density (g/cm ³)	0.8783	-	0.86-0.90	0.835
Viscosity (mm ² s ⁻¹)	4.415	1.9-6.0	3.5-5.0	3.34
Iodine value (I ₂ 100g ⁻¹)	58.3	-	120	55
Acid value (mgKOH/g)	0.08	0.50 max	0.50 max	0.1047
Cloud point (°C)	15.2	-	-	14
Pour point (°C)	15	-5 to -15	-	7

The characterization results of biodiesel produced from *Moringa* oil in this study are consistent with the findings of [22], who synthesized biodiesel from *Moringa oleifera* oil using an alkaline catalyst and evaluated it against similar standards. The viscosity of the biodiesel produced in this research met the required specifications and was comparable to the value reported by [23], who recorded 4.91 mm²/s. Likewise, the density of the biodiesel product showed minimal variation from that of the feedstock oil, which agrees with their observations.

5. CONCLUSION

The main limitation of this biodiesel lies in its pour point and cloud point. The low values of these parameters indicate poor cold-flow properties, making it less suitable for use in low-temperature environments. Despite this drawback, biodiesel from *Moringa* oil remains a promising and sustainable alternative to fossil fuels, as it is derived from a renewable, non-food-based feedstock that can be cultivated on marginal lands with minimal environmental impact. Promoting such sustainable biofuels is essential for reducing greenhouse gas emissions and achieving energy security.

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