

Research Article

In Situ Transesterification of Spirulina Microalgae to Produce Biodiesel Using Microwave Irradiation

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1. Introduction

The world needs energy to support economic and social progress and build a better quality of life, and in particular, more energy is required in developing countries [1]. For these reasons, the world is pursuing alternative renewable fuel sources to lessen the dependency on conventional fossil fuels.

First- and second-generation [2] biofuel resources have considerable economic and environmental limitations and do not seem to have the ability to fulfill the current requirement of biodiesel energy as would the third-generation biofuels from microalgae. The most common concern related to the current first-generation biofuels is that as production capacity increases, so does their competition with agriculture for arable land used for food production that may lead to severe food shortages [3]. In addition, the intensive use of land with high fertilizer and pesticide applications and water use can cause significant environmental problems [4]. The advent of second-generation biofuels was intended to produce fuels from lignocellulosic biomass, the woody part of plants that do not compete with food production. However,
converting the woody biomass into fermentable sugars requires costly technologies involving pretreatment with special enzymes, meaning that second-generation biofuels cannot yet be produced economically on a large scale [5]. Therefore, third-generation biofuels derived from microalgae according to Nigam and Singh [6] are considered a viable alternative energy resource that is devoid of the major drawbacks associated with first- and second-generation biofuels.

Microalgae have the distinctive ability to produce biodiesel in its natural form and thus avoid the requirements for complex, expensive processing methods/technologies, and there is no competition for agricultural land with other food crops as they grow or are cultivated in areas (marshy areas, brackish water areas, waste water, sewage, or marine environments) where crops do not grow [7].

Algae give potential benefits over lignocellulosic biofuel source crops as there is no competition for agricultural land with other food crops [8]. However, unlike vegetable oils from crops that can be mechanically extracted, releasing oil from algae cells is hindered by the rigid cell wall structure requiring solvent extraction [8]. This results in a high cost of oil recovery from microalgae prior to converting it into biodiesel. Other extraction methods such as supercritical fluid extraction, catalytic extraction, and ultrasonic extraction requiring longer extraction times large volumes of solvents, are energy and cost intensive [9].

Thus, microwave-assisted extraction or extractive transesterification could be an alternative to address the above concerns as the ability of the microwave to penetrate through the cell wall structure may result in an efficient recovery of lipids. Microwave irradiation leads to rapid generation of heat and pressure within the biological system, forcing out compounds from the biological matrix, producing good-quality extracts with better target compound recovery [10]. The rapid heating leads to localized high temperature and pressure gradients which assist in cellular wall degradation and enhanced mass transfer rates [11].

Most biodiesel is made by transesterification which involves mixing a lipid (triglyceride) with alcohol, in the presence of a catalyst, to produce esters and glycerol. A more common alcohol used is methanol which leads to the production of methyl esters, as shown in Equation (1) [12].

\[
\begin{align*}
&CH_2\text{−}OCOR_1 \quad R_1\text{COOCH}_3 \quad CH_2\text{OH} \\
&CH_2\text{−}OCOR_2 + 3CH_2\text{OH} \xrightarrow{\text{Catalyst}} R_1\text{COOCH}_3 \quad 3\text{CHOH} \\
&CH_2\text{−}OCOR_3 \quad R_1\text{COOCH}_3 \quad 3\text{CH}_2\text{OH}
\end{align*}
\]

\[(\text{Triglycerides}) \quad (\text{Methanol}) \quad (\text{Methyl ester}) \quad (\text{Glycerol})\]

\[(1)\]

Transesterification is a reversible reaction and is expected to occur in three steps: conversion of the long-chain triglycerides to diglycerides, conversion of diglycerides to monoglycerides, and finally the conversion of monoglycerides to esters [13].

Biodiesel can be produced from any vegetable oil (edible or nonedible), used, cooking oils, animal fats, and microalgae oils [14]. The best choice of a given feedstock for biofuel production should constitute a carbon chain length from the saturated C14 to C22, the unsaturated free fatty acid groups, and the saturation states which should be lower for better conversion [15]. The most important aspect considered is the chain length, whereas the usage of algae biodiesel is negatively affected by high unsaturation levels. The level of unsaturation affects negatively on oxidative stability, cetane number, and heat of combustion [15].

The biodegradability, nontoxicity, renewability, safe, and green form of alternative fuel and its low environmental impact make the Spirulina platensis algae a good feedstock for the production of alternative fuel [16]. Moreover, the use of these algae as suitable alternatives is because some species have high quantities of oil, which can be simultaneously extracted and transesterified into biodiesel and finally refined [17]. There is extensive literature carried out to explain biodiesel production from vegetable oils [18–21]. Yet, according to Demibras [22], there is little research carried out on the production of biodiesel from microalgae and especially regarding the feedstock Spirulina. Microalgae biodiesel production by conventional methods has been studied [17, 23–25], in which the oil is initially extracted mechanically and later by solvent extraction, followed by catalytic transesterification.

Algae oil has high free fatty acids (FFA), and the use of alkali catalyst leads to soap formation, increase in catalyst consumption, catalyst fouling, and decrease in the yield of biodiesel [17, 26]. Acid catalysts do not form soap but have slower reaction rates, taking a longer time for the reaction to be completed [26]. Sulphuric acid has been used as a catalyst in this study.

The in situ process simultaneously combines the steps of lipid extraction and transesterification to produce biodiesel and minimizes the cost of biodiesel production [27]. Deepalakshimi et al. [28] carried out an optimization process of biodiesel from waste avocado seeds by in situ method, and they obtained a yield of 94.4 wt% with respect to the weight of the total oil content of avocado seeds. El-Shimi et al. [17] produced biodiesel from Spirulina platensis microalgae by in situ method and stated that 8 hours was the optimum time required by conventional heating (using a hot plate).

The conventional heating consumes high amount of energy and has a long reaction time [29]. Microwave irradiation provides a rapid, energy efficient, cost-saving, and environmentally friendly method for the production of biodiesel. Other than for transesterification reactions, microwave energy also increases the rate of oil extraction to produce a greener and better quality biodiesel [30].

In the present study, biodiesel from Spirulina algae was produced by simultaneous extraction and transesterification by the use of microwave irradiation, to achieve a high degree of oil/lipid removal from Spirulina algae biomass with high efficiency of conversion to biodiesel [8].

2. Materials and Methods

2.1. Materials, Equipment, and Apparatus. Methanol, sulphuric acid, sodium hydroxide, hydrochloric acid, ethanol, and potassium hydroxide, all analytical grade, were sourced from Gelsup Kenya. Isopropyl alcohol, analytical grade, was
from Bevic, Kenya. The major equipment was as follows: microwave oven 1300 Watts (Shivaki, Japan), Stirrer 50 w (Bibby Sterilin Ltd., UK), rotary vacuum evaporator (HAHN-VAPOR, Hahnshin Scientific Co. Ltd., Korea), centrifuge (Itettich Zentrifugen D-7200 Tutlingen 6000 rpm, Germany), hot plate (Thomas Scientific, CAT no. 984THOCHPEUA), analytical balance, lab oven, and the Soxhlet apparatus (PYREX UK). *Spirulina* microalgae biomass, *Arthrospira platensis*, was obtained from Masinde Muliro University of Science and Technology, Kenya. Algae characteristics and growth conditions were as follows: filament average length and diameter 1,000 and 10 microns; lipid content 6-8% of dry biomass; growth nutrients (per litre of water), sodium bi-carbonate 16 g, potassium nitrate 2 g, common salt 1 g, magnesium sulphate 0.1 g, monoammonium phosphate 0.1 g, calcium chloride 0.1 g, potassium sulphate 0.1 g, iron sulphate 0.01, urea 0.02 g, and citric acid 0.02 g; pH 7; and average temperature 20.4°C.

2.2. Extraction and Characterization of Oil, Algae Biomass, and Biodiesel. A Soxhlet apparatus fitted with a quick-fit condenser and a 250 ml round bottom flask holding the solvent (a mixture of hexane/isopropyl alcohol, 3:2 to make a total of 120 ml) was used to extract oil from the algae biomass. The oil was separated from the solvent using a rotary vacuum evaporator. Oil yield of microalgae biomass (%) was calculated according to Equation (2) [17]:

\[
\text{Oil yield of microalgae biomass} = \left( \frac{\text{mass of the extracted oil}}{\text{mass of dried algae biomass}} \right) \times 100
\]  

Algae biomass was analysed for protein, ash, and moisture content by using ASTM standard methods. *Spirulina* oil was tested for moisture content, FFA, acid value, density, viscosity, and calorific value [31]. Similarly, specific gravity, kinematic viscosity, calorific value, acid number, flash point, carbon residue, ash content, S, pH, and moisture content of FAME were determined using standard methods according to American Standard Test Methods (ASTM D 67551) [31].

2.3. Transesterification Reaction. The experimental setup consisted of a batch reactor (250 ml round bottom flask) placed in a microwave oven, as shown in Figure 1. The flask was fitted with a reflux condenser and a glass rod for mechanical stirring. The weighed amount of *Spirulina platensis* microalgae biomass was mixed with measured amounts of H₂SO₄ catalyst and methanol. Blending of the mixture was carried out for a few minutes to achieve homogeneity before being poured into a reaction vessel. The reactants were then heated using microwave irradiation and maintained for a specified duration. After the reaction had taken place, the mixture was cooled in ice water to stop the reaction. The reactor contents were centrifuged at 3,000 rpm for a period of 30 minutes to separate biodiesel from glycerol and suspended solids. Biodiesel was decanted and washed a number of times to remove any acid catalyst, before being heated at about 100°C to remove any accompanying methanol and water. Biodiesel was analysed by GC for fatty acids. The experimental sequence for single-step (in situ) microwave-assisted extraction and transesterification process is illustrated in Figure 2.

This study optimized the process variables for the highest yield: algae to methanol ratio, catalyst concentration, and reaction time.

2.4. Design of Experiment for Optimization Studies. Response surface methodology (RSM) and central composite design (CCD) circumscribed [32] was used to optimize the
operational factors and maximize the production at five levels of the three variables under study requiring 20 sets of experimental runs consisting 9 factorial (cubic points), 5 axial (star points), and 6 replicates of centre points. These were used to analyse optimally the influence of various methodology process variables (catalyst concentration, methanol to oil ration, and reaction time on FAME yield) on the fatty acid methyl acid ester conversion and then finally determining its maximum productivity. Subsequent values that were acquired from the runs using the predicted optimal conditions were then used as the validating sets and were compared with the computed optimal values. A residual analysis was also carried out to verify the assessment of the model assumptions, namely, the evaluation of all important effects, normality distribution of noise, or the error generated by the experiment and the experimenter (random noise), and finally to verify if the errors have the same variance. Table 1 shows the actual levels of independent variables for the selected factors.

Table 1: Levels for independent variables for selected factors designed through CCD.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Units</th>
<th>Symbols</th>
<th>$-\alpha$ (-1.6818)</th>
<th>-1</th>
<th>0</th>
<th>1</th>
<th>$\alpha$ (1.6818)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyst concentration</td>
<td>wt%</td>
<td>$X_1$</td>
<td>0.32</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3.6818</td>
</tr>
<tr>
<td>Methanol ($CH_3OH$)</td>
<td>wt/vol</td>
<td>$X_2$</td>
<td>4.000</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>14.00</td>
</tr>
<tr>
<td>Reaction time</td>
<td>Min</td>
<td>$X_3$</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Table 2: Characterization of *Spirulina platensis*.

<table>
<thead>
<tr>
<th>Property</th>
<th>wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>7.8</td>
</tr>
<tr>
<td>Total lipid content</td>
<td>10.7</td>
</tr>
<tr>
<td>Protein content</td>
<td>51.83</td>
</tr>
<tr>
<td>Ash content</td>
<td>14.30</td>
</tr>
</tbody>
</table>

2.5. *Analysis of FAME*. Biodiesel was analysed for fatty acid methyl esters (FAME) using gas chromatography fitted with a flame ionization detector maintained at 260°C, the injector port at 240°C. The oven temperature program was as follows: temperature was held at 100°C for 2 min, increased to 140°C at 10°C/min, then 190°C at 3°C/min, increased to 260°C at 30°C/min, and finally maintained at 260°C for 2 minutes. Nitrogen gas was used both as carrier and make-up gas and allowed to flow inside a 30 m by 0.25 m by 0.25 μm Zebron ZB-FAME column fitted into the injector and detector ports inside the gas chromatography. Biodiesel sample (50 mg) to be eluted through the capillary column was mixed with 5 ml of methyl heptadecanoate ($C_{17}$ : 0, Gelsup 99%) to make a solution of 10mg/ml. The standard solution (methyl heptadecanoate, 2mg/ml) was initially prepared by dissolving in GC grade hexane (Gelsup, 98.9%). The required injection volume into the capillary column was 1 μl, which was used with the split ratio of 1 : 50 (EN-14214:2003). Fatty acid methyl ester content was calculated using the EN-14103 method.

![Diagram of in situ process of biodiesel production with CH₃OH recovery units.](image_url)
Table 3: Physicochemical properties of *Spirulina* microalgae oil.

<table>
<thead>
<tr>
<th>No.</th>
<th>Property</th>
<th>Test method</th>
<th>Limits ASTM D6751</th>
<th>Values of algal oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>—</td>
<td>7-9</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Specific gravity</td>
<td>ASTM D 941</td>
<td>0.86-0.89</td>
<td>0.877</td>
</tr>
<tr>
<td>3</td>
<td>Kinematic viscosity at 40°C mm²/s</td>
<td>ASTM D 445</td>
<td>1.9-6.0</td>
<td>5.76</td>
</tr>
<tr>
<td>4</td>
<td>Acid value KOH/g</td>
<td>ASTM D 664</td>
<td>—</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>Free fatty acid value</td>
<td>—</td>
<td>0.802</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Moisture content %</td>
<td>ASTM D 2709</td>
<td>0.050 max</td>
<td>7.8</td>
</tr>
<tr>
<td>7</td>
<td>Carbon residue %</td>
<td>ASTM D 524</td>
<td>0.050 max</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>8</td>
<td>Refractive index</td>
<td>—</td>
<td>1.365</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Sulphur, wt%</td>
<td>ASTM D 5453</td>
<td>0.0-0.0024</td>
<td>Nil</td>
</tr>
</tbody>
</table>

3. Results and Discussions

3.1. Characteristics of Algal Biomass and Oil. Characteristics of dried, bluish green *Spirulina* microalgae biomass in powder form (size 160 μm) is given in Table 2.

The total lipid content of microalgae biomass is greatly dependent on the specific growth conditions and the type of microalgae species in use, according to El Shimi et al. [33]. The microalgae culture conditions, nutrients, and light intensity can be optimized to increase the oil content of the biomass, leading to the increase in biodiesel production [33]. Microalgae grown under optimal conditions (right temperature, right pH (potential hydrogen), and correct amount of nutrients) produce large amounts of biomass but with low neutral lipid content, while microalgae grown in nutrient starvation (i.e., limiting nitrogen (N) and/or phosphorus (P)) accumulate high levels of neutral lipids but are slow growing [34].

3.2. Physicochemical Properties of *Spirulina platensis* Microalgae Oil. In the evaluation of the quality of the extracted *Spirulina* microalgae oil, tests were carried out as per the procedures stated in “Materials and Methods” to determine the physicochemical properties, and the results are as shown in Table 3. These properties are directly related to the yield and quality of the biodiesel produced. Kinematic viscosity and specific gravity were found to be 5.8 mm²/s at 313.15 K and 0.877, respectively, as obtained by El Shimi et al. [33]. These values show a good agreement with the reference values given in Table 3 and, along with other properties, confirm the good characteristics of *Spirulina* oil to produce biodiesel.

3.3. Fatty Acid Composition of *Spirulina platensis* Lipids and Lipid Classes (Relative Content in %). Table 4 gives the fatty acid composition of *Spirulina* microalgae oil obtained by GC. The main fatty acid components are linolenic (C18 : 3), Palmitic (C14 : 0), and linoleic acid (C18 : 2), respectively (see Table 4).

The percentage of saturated fatty acid present in biodiesel is 53.80 whereas the unsaturated is 46.20. The high fatty acid percentage contributions of the saturated palmitic (C14), both the unsaturated linoleic (C18 : 1), and linolenic (C18 : 2) fatty acids indicate good properties of *Spirulina* microal-gae in the production of biodiesel. Fatty acid detected in trace amounts and deemed insignificant was not included in the analysis. Fatty acid composition gave the average molecular weight of *Spirulina platensis* oil to be 849.80.

3.4. RSM for Optimization of the Process Variables. The central composite design matrix for RSM is given in Table 5.

3.4.1. ANOVA for Quadratic Model. Table 6 gives the ANOVA for regression analysis for microwave irradiation for a full quadratic model.

The model $F$ value of 170.10 implies the model was significant. There was only a 0.01% chance that an $F$ value this large could occur due to noise. $p$ values of “Prob.$>F$” less than 0.0500 indicate the model terms are significant. For catalyst concentration ($X_1$), the table $F$ value for alpha = 0.05 is 4.96, which was less than the calculated $F$ of 191.07 and $p < 0.05$; both indicate that the variable was significant and the null hypothesis was rejected. Similar observations hold for $X_3$, $X_3^2$, $X_2 X_3$, $X_1 X_3$, $X_1^2$, $X_2^2$, and $X_3^2$; all were significant model terms, and the null hypothesis was rejected. For the interaction $X_2 X_3$, the calculated $F$ was smaller and $p > 0.05$, indicating the term was not significant and the null hypothesis was valid. However, the term could not be dropped because it was part or supported by the model hierarchy.

The "lack of fit $F$ value" of 0.92 implies the lack of fit was not significant relative to the pure error. There was a 53.32% chance that a "lack of fit $F$ value" this large could occur due to...
noise. \( p \) value for lack of fit was >0.05 was nonsignificant which implies that there was no evidence that the model did not fit. The lack of fit was good.

The “Predicted \( R^2 \)” of 0.9700 was in reasonable agreement with the “Adjusted \( R^2 \)” of 0.9877, i.e. the difference is less than 0.2. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 31.945 indicated an adequate signal. This model can be used to navigate the design space. The model that predicts the percentage of FAME yield in terms of coded factors is given by Equation (3).

\[
Y_{\text{FAME}} \% = 80.29 + 7.07X_1 + 1.20X_2^2 + 6.85X_3 + 350X_1X_3 - 0.7423X_2X_3 - 7.18468X_1^2 - 14.3724X_2^2 - 7.72209X_3^2.
\] (3)
where \( X_1 \) refers to the actual catalyst concentration as a percentage of microalgae feed, \( X_2 \) refers to the volume of methanol to the algae biomass feed, and \( X_3 \) refers to the reaction time inside the microwave synthesis unit.

Equation (3) was then used to plot response surface and contours of FAME yield as an acid catalyst (\( \text{H}_2\text{SO}_4 \)) was taken into consideration. The relationships between yield and the three factors are shown in Figures 3–5.

### 3.4.2. Optimization and the Effects of Process Variables

#### 3.4.3. Effect of the Catalyst Loading

Figures 4 and 5 indicate the highest FAME yield corresponds to 2% catalyst concentration.

According to Prafulla et al. [9], lower concentration of the catalyst may not efficiently advance the reaction as the catalyst effect is hindered by the presence of a variety of organic compounds (lipids, olefins, fatty alcohols, phytols, and sterols) which may arise from algae biomass extraction [35]. When the catalyst ratio loading to dry microalgae biomass was increased further beyond 2.5%, the results obtained indicated a decrease in FAMEs content. This was brought about by the interaction of other molecules/compounds resulting in increased amounts of by-products. The addition of the catalyst in excess gives rise to the formation of an emulsion, which leads to an increase in viscosity and in the process to the formation of gels [36]. One other disadvantage of high catalyst concentrations, in general, is their corrosive nature which hinders the transesterification reaction [35].

#### 3.4.4. Effect of Methanol

The ratio of microalgae to methanol in use in the in situ transesterification of microalgae to produce FAME remains significant as the ratio increases from 1:4 to 1:12 (g/ml). The methanol used serves two purposes: it acts as an extraction solvent of the microalgae lipids and as reactant species for transesterification to take place [9]. However, according to Li et al. [37], an adequate amount of methanol is required for a higher yield of in situ biodiesel production processes because the methanol in use plays the role of both reactant and substance to submerge algae biomass. According to the results which are presented in Figure 4 and Table 5, 1:9 dry algae biomass to methanol (g/ml) was the most efficient feed ratio of the two variables under study. With the algae biomass to methanol (g/ml) increasing from 1:4 to 1:9, the FAMEs yield increased considerably. The FAME content achieved is clearly the highest at 1:9 dry algae biomass to methanol (g/ml) ratio. The higher the dry algae to methanol ratios above 1:12 (g/ml) does not favour the extraction and transesterification as much of the microwave energy will be absorbed by the solvent, and in the process having no effect on the algae biomass which may result in inefficient extraction of algae oils [9]. Increased amounts of solvent may also result in greater loss of solvent or aggravated rates of solvent recovery. Moreover, excessive methanol amounts may reduce the concentration of the catalyst in the reactant mixture and in the process retard the transesterification reaction [9].

#### 3.4.5. Effect of Reaction Time

Figures 3 and 4 show that the highest yield occurs in about 6-7 minutes of reaction time. Lower reaction times do not provide sufficient interaction of the reactant mixtures. Higher reaction time does not increase the conversion, but favours the backward reaction (hydrolysis of esters and thus the formation of glycerol),

![Figure 3: RSM plot: effect of volume of methanol (CH₃OH) and time on FAME yield.](image-url)
Figure 4: RSM plot: effect of catalyst concentration (H₂SO₄) and time on FAME yield.

Figure 5: RSM plot: effect of catalyst concentration (H₂SO₄) and volume of methanol (CH₃OH) on FAME yield.
which results in a reduction of product yield [38]. Additionally, having higher reaction times above 8 minutes may not favour sufficient conversion, as it may lead to loss of the solvent, overheating of the reaction mixture, by-product formation, and energy losses.

The optimal process conditions of the three variables chosen for the in situ process of biodiesel production as obtained from analysis above are dry *Spirulina* algae biomass to methanol ratio of 1:9 (g/ml), catalyst concentration of 2 (wt%), and the reaction time of 6-7 minutes.

### 3.5. Properties of *Spirulina* Microalgae Biodiesel

Table 7 shows the characteristics of the *Spirulina* microalgae biodiesel alongside European standards. These values show a good agreement with the information presented in Table 7, and along with these properties, the calorific value was similar to that presented by other researchers that includes a value of 32,911.34 kJ/kg confirming the characteristic of *Spirulina* biodiesel.

<table>
<thead>
<tr>
<th>No.</th>
<th>Property</th>
<th>Test method</th>
<th>Limits ASTM D6751</th>
<th>Values of algae biodiesel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>ASTM D-941</td>
<td>7-9</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Specific gravity</td>
<td>ASTM D 93</td>
<td>0.86-0.89</td>
<td>0.868</td>
</tr>
<tr>
<td>3</td>
<td>Flash point (°C)</td>
<td>ASTM D 445</td>
<td>1.9-6.0</td>
<td>4.45</td>
</tr>
<tr>
<td>4</td>
<td>Kinematic viscosity at 40°C (mm²/s)</td>
<td>ASTM D 240</td>
<td>0.050 max</td>
<td>32911.34</td>
</tr>
<tr>
<td>5</td>
<td>Acid value (KOH/g)</td>
<td>ASTM D 664</td>
<td>—</td>
<td>0.475</td>
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<td>Free fatty acid value</td>
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<td>0.050 max</td>
<td>0.045</td>
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<tr>
<td>7</td>
<td>Calorific value (kJ/kg)</td>
<td>ASTM D 482</td>
<td>0.0001</td>
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<td>Moisture content (%)</td>
<td>ASTM D 2709</td>
<td>0.050 max</td>
<td>0.001</td>
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<tr>
<td>9</td>
<td>Carbon residue (%)</td>
<td>ASTM D 5453</td>
<td>0.0-0.0024</td>
<td>Nil</td>
</tr>
<tr>
<td>10</td>
<td>Ash content mass %</td>
<td>ASTM D 5453</td>
<td>0.0-0.0024</td>
<td>Nil</td>
</tr>
</tbody>
</table>

### 4. Conclusions

*Arthospira Spirulina platensis* microalgae oil was transesterified with methanol and acid catalyst, and a high yield of 83.4% of FAME was obtained. The in situ reaction used algal biomass, eliminating the expensive solvent extraction, and microwave irradiation reduced the reaction time to less than 7 minutes. Conventional heating takes about 3 hours and above for similar yields. FAME produced met the requirements to be used as a diesel fuel.

### Data Availability

The article contains all the relevant data. The corresponding author would provide any additional data upon request.

### Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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