TRENDS IN SCIENCES 2026; 23(3): 11489
https://doi.org/10.48048/tis.2026.11489 RESEARCH ARTICLE

Optimizing Biodiesel Yield from Microalgae: A Comparative Study of *Spirulina* sp. and *Nannochloropsis oculata* Using KOH-Catalyzed *In-Situ* Transesterification

Nita Ariestiana Putri[1,*], Resti Nurmala Dewi[2], Benny Arif Pambudiarto[3], Jefri Pandu Hidayat[1], Nie Luhh Dhian Forestry[1], Alvin Nur Rahman[1], Rizka Lestari[1], Muhamad Maulana Azimatun Nur[4], Ira Nurhayati Djarot[5], Asful Hariyadi[1] and Elizabeth Desfelia Ciciolini Sidabutar[1]

[1]*Department of Chemical Engineering, Institut Teknologi Kalimantan, Balikpapan, Indonesia*
[2]*Department of Marine Product Processing, Polytechnics of Marine and Fisheries of Jembrana, Jembrana, Indonesia*
[3]*Department of Chemical Engineering, Faculty of Engineering, Universitas Muhammadiyah Gresik, Gresik, Indonesia*
[4]*Department of Chemical Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta, Yogyakarta, Indonesia*
[5]*Research Center of Sustainable Production System and Life Cycle Assessment, National Research and Innovation Agency (BRIN), Kawasan Sains dan Teknologi (KST) BJ Habibie, (PUSPIPTEK), Puspiptek Street, Serpong, South Tangerang, Banten, Indonesia*

([*]Corresponding author's e-mail: nita.ariestiana@lecturer.itk.ac.id)

Received: 4 August 2025, Revised: 22 August 2025, Accepted: 10 September 2025, Published: 30 November 2025

Abstract

The increasing demand for renewable fuels necessitates low-cost, high-yield biodiesel feedstocks. This study investigates biodiesel production from *Spirulina* sp. and *Nannochloropsis oculata* cultivated in 20% tofu wastewater, integrating waste valorization with sustainable biomass generation. Biodiesel was produced using in-situ transesterification, a single-step method that combines lipid extraction and conversion directly from wet biomass, with potassium hydroxide as a catalyst. The research aimed to optimize 2 key parameters: methanol-to-biomass ratio weight by volume (3:1 until 7:1 w/v) and reaction time (30 - 120 min). Wet biomass was reacted with methanol and 2% KOH under controlled stirring and temperature conditions (80 °C). The optimal conditions for highest biodiesel yields were obtained at a methanol-to-biomass ratio of 7:1 and 120-min reaction time: 12.5% for *Spirulina* and 17.5% for *Nannochloropsis oculata*. Kinetic analysis revealed solvent ratio significantly enhanced transesterification efficiency. Nannochloropsis oculata performed better at low ratios, *Spirulina* sp. at higher ratios, confirming biodiesel feasibility from tofu wastewater-based microalgae. Fourier Transform Infrared Spectroscopy (FTIR) spectra confirmed the presence of ester functional groups, while Gas Chromatography Mass Spectrometry (GCMS) analysis identified key fatty acid methyl esters such as methyl palmitate and methyl oleate. *Nannochloropsis oculata* biodiesel exhibited a greater proportion of saturated Fatty Acid Methyl Esters (FAMEs), indicating better fuel feedstock. *Nannochloropsis oculata* biodiesel exhibited higher saturated FAME content. These results establish the technical feasibility of this approach and underscore its potential contribution to future biofuel development. Nonetheless, comprehensive physicochemical and performance evaluations are required to fully validate its application as a commercial biodiesel alternative.

Keywords: Biodiesel, Fatty Acid, *In Situ* Transesterification, KOH Catalyst, *Nannochloropsis oculata*, *Spirulina* sp.

Introduction

An important factor driving the increase in energy demand is the rising population and improve living standards, especially in low- and middle-income countries. This has an impact on petroleum fuels which are expected to continue to decrease if continuously used and exploited. Moreover, the combustion of fossil fuels has detrimental impacts on the environment due to the release of greenhouse gases and other harmful emissions [1]. These pollutants not only contribute to global environmental issues such as air pollution and climate change, but also pose serious risks to human health, including respiratory diseases, asthma, and ocular disorders [1]. Currently, the world's fuel energy needs, including Indonesia, are still heavily dependent on fossil fuels [2]. However, the availability of this resource is expected to continue declining in the future. Therefore, the development of renewable fuels is crucial for maintaining the sustainability of energy supply. Biofuels such biodiesel offer a sustainable alternative to fossil fuels by providing renewable energy with lower greenhouse gas emissions and reduced environmental impact [1,3]. The fundamental necessities of biodiesel are that it should to be created from renewable crude material, and have a lower negative natural effect than that of fossil fuels [4] . Biodiesel energy can be used as a renewable energy source to balance the increasing oil consumption.

Biodiesel is an alternative fuel derived from renewable natural resources, such as waste cooking oils [5], vegetable seed oils [6], animal fats [7] and microalgae oils [8]. It is specifically designed for diesel engines and consists of mono-alkyl esters derived from long-chain fatty acids [9]. Biodiesel has recently gained attention in the development of alternative fuels because of its great potential and properties of being renewable, biodegradable, non-toxic, sulfur-free, having a higher flash point and producing cleaner emissions [10]. The limited supply of raw materials is one of the main obstacles in biodiesel production.

The high lipid content and rapid growth rates of microalgae make them a viable feedstock for the generation of biodiesel [11]. Koech *et al.* [1] added that algae have a potential to produce maximum biodiesel. A previous study found that, *Spirulina* sp. has an (Fatty Acid Methyl Ester) FAME yield of 99% under optimal conditions at 80◦C temperature, and a 30:1 methanol/oil molar ratio [12]. Research conducted by Davoodbasha *et al.* [13] showed that microalgae *Chlorella vulgaris* was able to produce a FAME yield of 67% under optimal conditions at 80 °C temperature, a 9:0.6 methanol/oil molar ratio, with a reaction period of 4 h. Meanwhile, Pradana *et al.* [14] found that reacting *Spirulina* sp. with methanol and palm oil using KOH as a catalyst in a single-step process produced 85.27% biodiesel under optimal conditions. This method increased the yield by 34.59% compared to the conventional 2-stage process and reduced unsaturated fatty acids, thereby enhancing the fuel quality. Other studies have also shown that microalgae strains have the potential to be a source of biodiesel, namely *Nannochloropsis* sp. [15-17]. Treatment of *Nannochloropsis gaditana* with methanol produced a FAME yield of 87.25% [18]. Mirzayanti *et al.* [19] reported that the highest yield (17.8%) was obtained by *Nannochloropsis* sp. under raw material ratio of 1:25, using 4 wt% CaO and a reaction time of 20 min. Meanwhile, Purkan *et al.* [20] found that *in situ* transesterification of *Nannochloropsis* sp. oil achieved a biodiesel conversion yield of 89.72%.

In Indonesia, efforts to develop microalgae as a renewable energy source are ongoing, including the identification of various microalgae species that thrive in tropical regions [21]. Microalgae are single-celled plants that undergo photosynthesis, utilizing nutrients to produce biomass and secondary products such as proteins, carbohydrates, and lipid under certain environmental conditions [22]. Microalgae, which are known for their lipid content and classification as aquatic plants, have significant advantages over other fuel sources. They do not require extensive cultivation areas, produce biomass very quickly, and can reduce air pollution by utilizing CO_2 for growth [23].

Microalgae exhibit high photosynthetic conversion efficiency, thrive in diverse ecosystems, possess a rapid biomass growth rate, and serve as a promising source of biodiesel feedstock [24]. They can be cultivated in seawater, saline water, fresh water, and even wastewater [25]. Mohsenpour *et al.* [26] demonstrated that microalgae cultivation, utilizing organic wastewater as a growth medium, offers a safer and more easily re-neutralized process. Feng *et al.* [27], reported that liquid waste from the tofu industry

contains significant amounts of carbon, nitrogen, and phosphorus making it a promising medium. Higher biomass and lipid content were observed in *Chlorella pyrenoidosa* grown in tofu wastewater than in BG-11 medium, as reported by [28]. Similarly, Elystia *et al.* [29] observed that *Scenedesmus* sp. cultivated in 20% tofu wastewater yielded higher biomass (6.63×10^6 cells/mL) and lipid content (29.73%) compared to the control. Dewi *et al.* [30] also mentioned that optimal growth of *Botryococcus braunii* was observed in 10% tofu liquid waste, yielding a biomass concentration of 6.57 ± 0.03 g/L and a lipid content of 41.13% ± 0.74% compared to control. Tofu wastewater was also successfully utilized by microalgae to produce lipid namely *Chlorella vulgaris* (23%) [31], *Botryococcus braunii* (0,8716 g/L) [32], *Chromochloris zofingiensis* (30.49%) [33], *Nostocmuscorum* (6.65 ± 0.16 mg/g) [34], *Chlorella pyrenoidosa* (254.9 mg/L/day) [28]. In a study by Putnarubun *et al.* [35] the microalgae species *Tetraselmis* sp., grown in tofu wastewater, yielded 8.6% oil composed of palmitic, stearic, and oleic acid, which was then converted into biodiesel via indirect transesterification with an acid number of 4.14 mg KOH/g, a viscosity of 2.6 cSt, and a density of 2.6 g/cm³.

Spirulina sp. and *Nannochloropsis oculata* are 2 other microalgae that can be used to produce biodiesel and may be cultivated in tofu wastewater [36]. These strains have several advantages, including very high nutrition, ease of mass cultivation, abundant availability, and relatively fast growth [25]. *Spirulina* sp. is a species of blue green algae with high protein content and, contain amino acids, lipids, fatty acids, carbohydrates, vitamins, and minerals [37,38]. According to Pradana *et al.* [14], *Spirulina* sp. contains 0.25% lipid, 44.72% protein, and 46.13% carbohydrate. Similarly, Taiti *et al.* [39] reported that *Spirulina* sp. is composed of 0.27 ± 0.01% lipid, 37.90% carbohydrate, and 54.84% ± 0.13% protein (per 100 g dried biomass). In contrast, Prasetyo *et al.* [40] found that *Spirulina* sp. contains 2.09% lipid, 54.39% protein, and 3.21% fiber. On the other hand, *Nannochloropsis oculata* has a fairly high fat content with a total amount ranging from 31% to 68% dw [41,42]. Banarjee *et al.* [43] reported that *Nannochloropsis oculata* contains 13.02% ± 0.91% lipid, 32.82 ± 3.11% protein, and 26.13 ± 2.70% carbohydrate.

Transesterification is a key technique in the biodiesel industry, and has been widely applied. Traditionally, biodiesel production involves lipid extraction from microalgae, followed by transesterification (ex situ transesterification). However, this method is time consuming and complicated, making it economically impractical [44]. In addition, this conventional method has disadvantages such as the need for high temperature, reaction time, use of large amounts of solvent, and difficulty in production scale adjustment [45]. The in-situ transesterification method is a direct approach to processing biomass containing many lipids without having to go through the extraction and purification stages separately again [17]. Pradana *et al.* [14] reported that in-situ transesterification enhances process efficiency while minimizing chemical usage. Similarly, Ideris *et al.* [46] highlighted that this approach has been demonstrated as both effective and economical for microalgae. In-situ transesterification produces a biodiesel yield that is comparable to that of the traditional 2-step procedure of lipid extraction followed by transesterification. Moreover, Thanh *et al.* [47] noted that this method is economically advantageous, with up to 90% energy savings since it eliminates the need for drying and oil extraction steps. In this method, the feedstock directly interacts with the alcohol and catalyst. Alcohol is used in the transesterification process as a reactant as well as a solvent for the oil.

Recent studies have explored biodiesel production from microalgae via in-situ transesterification. Cercado *et al.* [48] compared alkali catalysts, including KOH, NaOH, and LiOH, and found that transesterification of microalgae using KOH consistently produced superior FAME yields. The KOH/Al_2O_3 catalyst (35 wt% KOH) achieved an impressive 89.5% biodiesel yield from *Chlorella vulgaris in situ*, under conditions of 10 wt% catalyst, a methanol-to-biomass ratio of 8 mL/g, 60 °C, and 5 h [49]. In the direct *in situ* transesterification of *Chlorella vulgaris*, the optimized KOH and methanol concentrations yielded 85.6% biodiesel using a single-step base-catalyzed method at 60 °C [50]. However, there has been limited research examining the potential of *Spirulina* sp. and *Nannochloropsis oculata* as raw materials for biodiesel cultivation in tofu liquid waste using in-situ transesterification with KOH as a catalyst.

The present study aimed to evaluate the effect of incorporating tofu liquid waste into microalgae cultivation medium for biodiesel production, specifically considering the optimal methanol-to-biomass ratio and reaction time.

Materials and methods

In-situ transesterification materials

Biodiesel was produced from *Spirulina* sp. and *Nannocloropsis oculata* via an insitu transesterification method with a KOH catalyst. Both strains were provided by the Brackish Water Cultivation Fisheries Center, Jepara, Indonesia, and were cultivated in 20% (v/v) tofu wastewater for 14 d. Meanwhile, tofu liquid waste was supplied by a small-medium enterprise in Indonesia and contains 971.9 mg/L BOD5, 2,776.85 mg/L COD, 96 mg/L total N, 1,68 mg/L total phosphate [36]. Methanol (96 wt%, Merck Fulltime) obtained from UD SIP was used as the solvent. Additionally, potassium hydroxide (Merk SAP), also supplied by UD SIP, was utilized to accelerate the transesterification process. The experimental setup used in this study is shown in **Figure 1**.

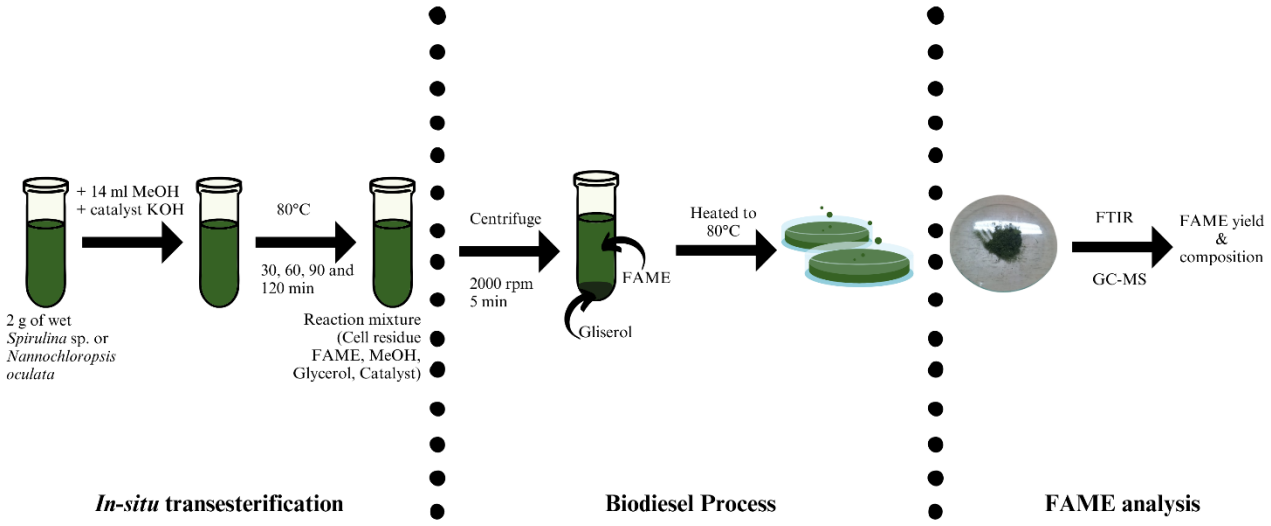

Figure 1 Method for *in-situ* transesterification of biodiesel from microalgae.

Biomass production

Spirulina sp. and *Nannochloropsis oculata* were cultivated in a photobioreactor, following the methodology established by Putri [36]. Cultivation parameters included a light intensity of 5,000 lx, a pH range of 8 - 9, temperatures between 20 and 30 °C, and a medium salinity of 30 ppt, maintained for 14 days. Subsequently, microalgae were harvested by adding 1 N NaOH to bind the biomass above the surface, forming flocs until sedimentation occurred at the bottom of the container by utilizing the force of gravity, following the method described by Dewi [51]. After standing for 24 h and sedimentation, the biomass was filtered using a filter cloth so that the biomass would be retained on the filter. The biomass of *Spirulina* sp. and *Nannochloropsis oculata* contained 90.41% and 86.55% moisture, 4.61% and 6.11% ash, 1.71% and 1.50% protein, and 2.44% and 1.50% lipid, respectively [36].

Experimental set-up

Wet biomass from each microalgae was subjected to in-situ transesterification to produce biodiesel, following the method described by Mirzayanti *et al.* [17] , with some modifications. In this process, methanol was used as the solvent with methanol-to-biomass ratios of 7:1, 5:1, and 3:1 (w/v). For each variable, 2 g of wet biomass was used. The transesterification process employed a 2% potassium hydroxide (KOH) catalyst and was performed at a stirring speed of 500 rpm. The reaction times were 30, 60, 90 and 120 min, and the temperature was maintained at approximately 80 °C. After the reaction, the mixture was centrifuged at 2,000 rpm for 5 min. The resulting liquid phase was then heated to approximately 80 °C. Biodiesel was weighed repeatedly until a constant mass was achieved. The final stable mass was recorded for each variable and used to calculate the biodiesel yield. Biodiesel yield (%) was determined using the following equation [52]:

$$\text{Yield of biodiesel (\%)} = \frac{\text{weight of biodiesel (g)}}{\text{weight of biomass (g)}} \times 100\% \quad (1)$$

Biodiesel analysis using Fourier transform infrared spectroscopy (FTIR)

The biodiesel microalgae sample was weighed with 100 mg KBr as much as 1 - 5 mg, then grind to form a pellet. Scanning was carried out 50 times with a wave number of 4,000 - 400 cm^{-1} at a resolution of 4 cm^{-1} and was recorded in the form of absorbance/transmittance. The FTIR spectra were analyzed using a Thermo Nicolet IS 10.

Fatty acid analysis using gas chromatography mass spectrometry (GCMS)

The biodiesel sample contained in the microtube was dissolved using the designated solvent (MeOH). The mixture was then vortexed until complete dissolution was achieved. If turbidity was observed, the solution was centrifuged at 900 rpm for 3 min. Subsequently, the supernatant layer was collected and injected into the GC-MS system with the injector maintained at 240 °C for 60 min.

Mathematical modelling

Mathematical modelling in this study aims to observe the distribution of compound concentrations in the biodiesel production process at each time point and determine the values of the constants in the mathematical equation. Assumptions used in this mathematical modelling:

1) Elementary kinetic equations are used to determine the reaction rate equation.

2) Stirring is carried out thoroughly so that the reaction is considered to occur homogeneously

Production of biodiesel consists of 3 consecutive reactions as shown by Eqs. (2) to (4):

$$\text{TG} + \text{MeOH} \overset{k_1,k_2}{\longleftrightarrow} \text{DG} + \text{FAME} \quad (2)$$

$$\text{DG} + \text{MeOH} \overset{k_3,k_4}{\longleftrightarrow} \text{MG} + \text{FAME} \quad (3)$$

$$\text{MG} + \text{MeOH} \overset{k_5,k_6}{\longleftrightarrow} \text{G} + \text{FAME} \quad (4)$$

Biodiesel production in the in-situ transesterification process occurs in 3 stages using 2 raw materials, namely triglycerides (TG)/lipids derived from microalgae and methanol (MeOH). The 1st stage is the reaction between TG and MeOH, producing diglycerides (DG), which are intermediate products, and biodiesel (FAME). The reaction rate equation is given in Eq. (5). The 2nd stage involves the reaction between the intermediate product DG and MeOH, producing monoglycerides (MG) and FAME. The reaction rate equation for this process is given in Eq. (6). The final stage involves the reaction between the intermediate product MG and MeOH, producing glycerol (G) and FAME. The reaction rate equation is given in Eq. (7).

$$r_1 = -k_1[TG][MeOH] + k_2[DG][FAME] \quad (5)$$

$$r_2 = -k_3[DG][MeOH] + k_4[MG][FAME] \quad (6)$$

$$r_3 = -k_5[MG][MeOH] + k_6[G][FAME] \quad (7)$$

where r_1, r_2, and r_3 represent the rates of reactions (1) to (3), respectively. In this study, the rate constants are expressed as functions of ratio and time reaction to investigate their influence on biodiesel yield. Based on mass balance in a batch reactor, the governing equation of concentration distribution is obtained as Eq. [53]:

$$r_i = \frac{1}{V}\frac{dN_t}{dt} = \frac{dC_i}{dt} \quad (8)$$

And by including the reaction rate equation for each compound, the following equation is obtained:

$$\frac{d[TG]}{dt} = -k_1[TG][MeOH] + k_2[DG][FAME] \quad (9)$$

$$\frac{d[DG]}{dt} = -k_1[TG][MeOH] - k_2[DG][FAME] - k_3[DG][FAME] + k_4[MG][FAME] \quad (10)$$

$$\frac{d[MG]}{dt} = -k_3[DG][MeOH] - k_4[MG][FAME] - k_5[MG][FAME] + k_6[G][FAME] \quad (11)$$

$$\frac{d[FAME]}{dt} = k_1[TG][MeOH] - k_2[DG][FAME] + k_3[DG][FAME] - k_4[MG][FAME] + k_5[MG][FAME] - k_6[G][FAME] \quad (12)$$

$$\frac{d[G]}{dt} = k_5[MG][MeOH] - k_6[G][FAME] \quad (13)$$

$$\frac{d[MeOH]}{dt} = -\frac{d[FAME]}{dt} \quad (14)$$

There are 6 constants in the entire equation. Simulation using MATLAB was performed to obtain the values of these constants.

Data analysis

Quantitative and qualitative data were obtained from biodiesel yield measurements and GC-MS and FTIR analyses. The collected data were subjected to descriptive analysis, and the results are presented in the form of graphs and tables to facilitate interpretation. Meanwhile, the kinetic modeling was conducted using MATLAB R2021a and Statistics Toolbox Release 2021b, The MathWorks, Inc., Natick, Massachusetts, United States.

Results and discussion

The effect of methanol to biomass ratio and reaction time on biodiesel yield

The effect of the methanol-to-biomass molar ratio weight by volume (3:1, 5:1, and 7:1 w/v) on the biodiesel yield was investigated at various reaction times (30 - 120 min) under constant conditions, including 2 wt% catalyst and a temperature of 60 °C, as shown in **Figure** 2 Effect of molar **methanol-biomass ratio and** reaction time on yield of biodiesel. In general, increasing the methanol ratio and reaction time had a positive effect on the biodiesel yield of both microalgae species. However, the performance of each microalgal species differed under each reaction condition.

Overall, these findings suggest that the 2 critical factors influencing transesterification efficiency are the reaction duration and methanol-to-biomass ratio. Particularly, for *Nannochloropsis oculata*, the maximum biodiesel output was obtained at a ratio of 7:1 and a reaction duration of 120 min. This finding is in line with previous studies by Kamyab *et al.* [54] and Boro *et al.* [55] who mentioned that a higher methanol ratio can accelerate the transesterification reaction rate and induce a shift in the reaction equilibrium towards the product (biodiesel) until it reaches the saturation point.

This is also in accordance with the result of Surendhiran *et al.* [56] research using *Chlorella salina* who found that the higher the methanol ratio, the higher the biodiesel yield. A higher molar ratio is required to increase the miscibility and enhance the contact between the alcohol molecule and triglyceride [57,58]. Kamel *et al.* [59] also explained that as the reaction time increases, the results obtained increase because the methanolysis process becomes more optimal. The increase in conversion is influenced by the duration of the reaction, which determines the extent to which interactions occur between the active sites of the catalyst, methanol, and Alga Oil (AO).

According to this study, the use of a KOH catalyst may accelerate the transesterification reaction and yield more esters in less time. As the reaction time increased, the heightened interaction between biomass, methanol, and the catalyst accelerated the conversion of triglycerides into biodiesel (methyl esters) and glycerol, leading to more frequent molecular collisions [60]. The KOH catalyst functions effectively when there is sufficient reaction time for the transesterification process to reach its peak, at which point all the constituents react and a significant amount of biodiesel is produced.

However, the yield of biodiesel in this is relatively low. This could be attributed to several factors. One possible reason is the use of a base catalyst (KOH). As noted by Thanh *et al*. acid catalysts have been reported to perform better in terms of FAME yield compared to base catalysts. When base catalysts are applied, they may react with free fatty acids (FFA) to form soap, which subsequently lowers the biodiesel yield [61]. In addition, the solvent system employed in this study consisted solely of methanol without the use of a co-solvent. Since methanol is polar and oils are nonpolar, the miscibility between the 2 is limited, leading to poor interaction between the reactants [62]. This phase separation slows down the reaction rate, reduces mass transfer efficiency, and results in incomplete conversion of triglycerides into biodiesel. The use of a co-solvent, however, has been shown to improve miscibility, enhance mass transfer, accelerate the reaction, and increase biodiesel yield, while remaining simple to remove and recycle after the process [64,65].

Another contributing factor is the high moisture content in the microalgal biomass, with *Spirulina* sp. containing 90.41% and *Nannochloropsis oculata* containing 86.55% water. Numerous studies have demonstrated that high water content negatively affects the transesterification process. Reports on *Chlorella* sp. and *Chlorella sorokiniana* showed that in-situ transesterification of dry biomass (0 wt% moisture)

resulted in FAME yields of 94% and 61% [65], whereas biomass moisture contents above 20% significantly reduced biodiesel recovery [66]. Cao *et al.* [67] further reported that biodiesel yield declined drastically from 91.4% to 10.3% as moisture content increased from 0% to 90%. The presence of excess water increases the energy required for the reaction to proceed effectively. Similarly, Wahlen *et al.* [68] found that using 100 mg of microalgal biomass with 400 mg of distilled water (80% water content) produced only 54% of the expected FAME yield under reaction conditions of 5 mL methanol, 1.8% (v/v) H_2SO_4, 80 °C, and 20 min of reaction time.

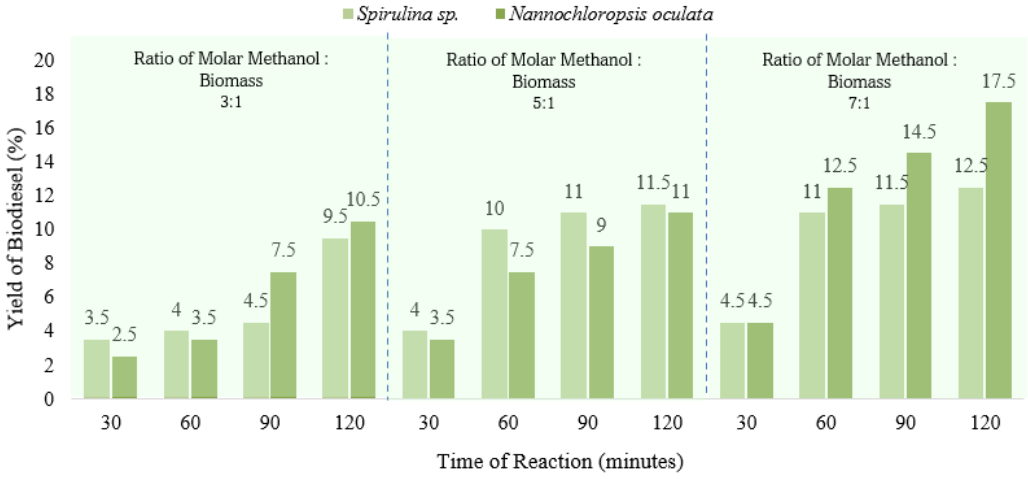

Figure 2 Effect of molar methanol-biomass ratio and reaction time on yield of biodiesel.

Comparison between of model and experiments with model evaluation

The following are the results of the simulation of the constant value finding **Figure 3**. Based on the values obtained, it can be concluded that the greater the ratio of solvent used, the faster the reaction rate will be in general, which implies that the required reaction time decreases accordingly. As presented in **Table 1**, the rate constants (k) consistently increased with higher methanol-to-biomass ratios, confirming that solvent ratio plays a critical role in enhancing transesterification efficiency for both *Spirulina* sp. and *Nannochloropsis oculata*.

The very small values of k_2, k_4, and k_6 indicate that the reaction tends to be irreversible, predominantly directed toward product formation rather than reverting to the reactants. Among all stages, k_1 and k_3 exhibited relatively higher values, suggesting that the dominant reaction pathways involve the conversion of triglycerides (TG) into diglycerides (DG) and monoglycerides (MG), followed by the formation of fatty acid methyl esters (FAME). Conversely, the conversion of TG to DG and FAME serves as the rate-determining step, as this stage presents the lowest constant values compared to other processes.

In comparing the 2 microalgae, there is no significant difference in their overall kinetic behaviour. At a 3:1 ratio, *Nannochloropsis oculata* showed slightly higher k_1 and k_3 values, while *Spirulina* sp. exhibited improved constants at higher ratios (5:1 and 7:1). As shown in **Table 1**, the highest rate constant observed was $k_5 = 0.6213$ mL·mol^{-1}·min^{-1} for *Spirulina* sp. at a 3:1 solvent-to-oil ratio, indicating a significantly faster reaction step compared to *Nannochloropsis oculata*, where the maximum k_5 value was 0.1094 mL·mol^{-1}·min^{-1}, highlighting the superior reactivity of *Spirulina* sp. under these experimental conditions. These findings demonstrate that both microalgae are equally promising feedstocks for biodiesel production, with reaction kinetics strongly governed by solvent ratio optimization and the TG-to-DG/FAME conversion step.

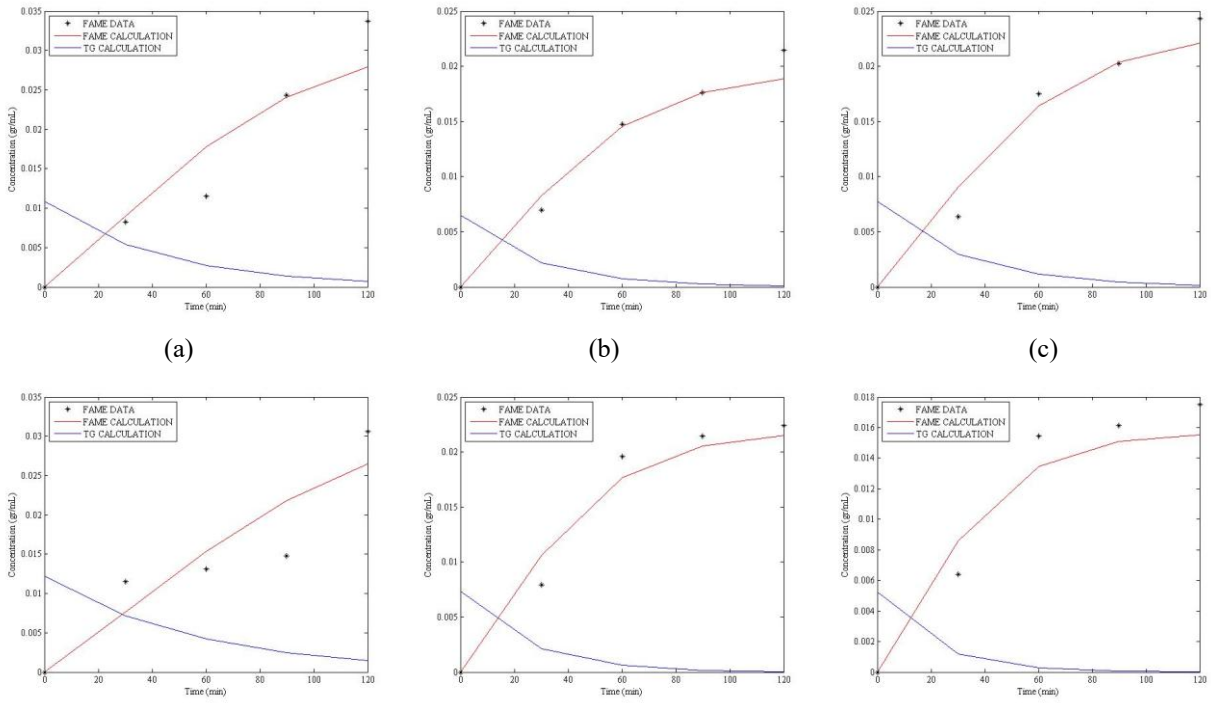

Figure 3 Experimental and predicted value of biodiesel concentration of *Spirulina* sp. and *Nannochloropsis oculata* with ratio of methanol: biomass (a) 3:1 *Nannochloropsis oculata*; (b) 5:1 *Nannochloropsis oculata*; (c) 7:1 *Nannochloropsis oculata*; (d) 3:1 *Spirulina* sp.; (e) 5:1 *Spirulina* sp.; (f) 7:1 *Spirulina* sp.

Table 1 Rate constant reaction of *Spirulina* sp. and *Nannochloropsis oculata.*

Rate constant reaction ($mL.mol^{-1}.min^{-1}$)	*Spirulina* sp.			*Nannnochloropsis oculata*		
	3:1	**5:1**	**7:1**	**3:1**	**5:1**	**7:1**
k_1	0.0223	0.053	0.0636	0.0299	0.0469	0.0412
k_2	0	0	0	0	0	0
k_3	0.0266	0.0812	0.0896	0.0842	0.0695	0.0674
k_4	0.0009	0	0	0	0.0001	0
k_5	0.6213	0.1262	0.1329	0.0758	0.1065	0.1094
k_6	0.0003	0	0	0	0	0

Identification of biodiesel function groups using FTIR

Biodiesel is a renewable fuel oil derived from plant-derived vegetable oils extracted from plants. Biodiesel consists mostly of fatty acid esters, which can be obtained from both vegetable oils and animal fats [9]. The in-situ transesterification method is a the biodiesel manufacturing process that refers to the direct use of biomass without conducting a lipid extraction process first [69]. Methyl esters are compounds produced through the transesterification process, in which the reaction occurs between fatty acids and alcohols, especially methanol. In biodiesel production, methyl ester is the main component formed from the reaction between triglycerides in feedstocks, such as microalgae and methanol [70]. The compounds expected to be present in the microalgal biodiesel content in this FTIR analysis indicate that the compounds are esters, characterized by the presence of C=O or C-O functional groups. This analysis was based on previous studies by Elgharbawy *et al.* [70], which showed spectral patterns typical of microalgal biodiesel.

The presence of C=O and C–O groups thus provide clear evidence of ester formation, confirming biodiesel

production. Based on the study conducted by Arif *et al.* [71], a more targeted approach was applied by aligning the FTIR spectra with the specific biochemical characteristics of microalgal biomass. The spectra showing peaks at 1,750 - 1,720 cm^{-1} and 1,163 - 1,210 cm^{-1} were identified as clear signals of ester groups derived from triglycerides and fatty acids, which serve as key indicators of biodiesel formation. In addition, absorption bands appearing at 3,100 - 2,800 cm^{-1} and 2,830 - 2,695 cm^{-1} were associated with alkane and aldehyde groups, respectively. The presence of these bands illustrates the transformation of organic compounds in microalgal biomass into biodiesel molecules.

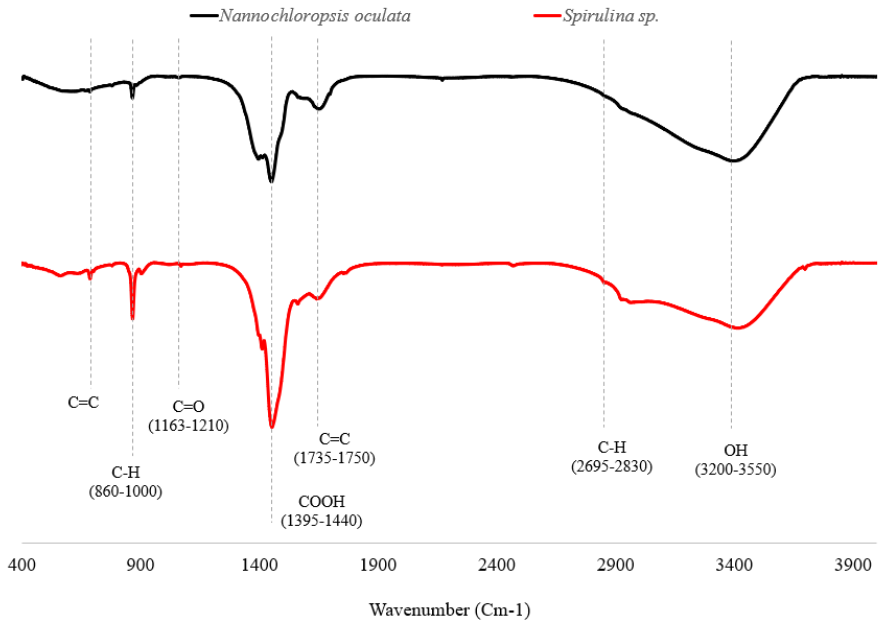

Figure 4 FTIR spectrum of biodiesel from *Spirulina* sp. and *Nannochloropsis oculata.*

The FTIR spectra of the biodiesel derived from Spirulina sp. and Nannochloropsis oculata are shown in **Figure 4** *and*

Table *2* Comparing the FTIR wavelengths of biodiesel derived from Spirulina sp. and Nannochloropsis

Table *2*. In both *Spirulina* sp. and *Nannochloropsis oculata*, a wide band at wave number 3,415 cm^{-1} of moderate intensity (br,m) at 3,415 cm^{-1} was observed. This band is attributed to the O-H stretching band of the hydroxyl groups that could be associated with the free alcohol, unreacted water, or residual methanol reagents, which did not undergo a complete transesterification reaction. These groups also frequently appear as minor contaminants in microalgal biodiesel. These findings are supported by research conducted by Sukarni [72] which occurred in the absorption region of water content at wave numbers of 3,343 and 3,645 cm^{-1}. This is related to the O-H stretching of water absorbed by the silica contained in the ash, as well as strong, medium, and weak features in the fingerprint region associated with other inorganic components remaining in the ash.

The presence of long aliphatic chains is indicated by the strong absorption bands at 2,923 - 2,925 cm^{-1} and 2,853 - 2,854 cm^{-1}, representing the asymmetric and symmetric stretches of the methylene group (CH_2), respectively, which are commonly found in fatty acids as the main precursors of biodiesel [73]. The most prominent characteristic band is located at 1,743 cm^{-1} with a very strong intensity (vs), which indicates the carbonyl stretch (C=O) of the ester group. This is a key indicator of the formation of methyl esters, the main compound in biodiesel, as confirmed by Andreo-Martínez *et al.* [7] and Taiti *et al.* [39]. Another band at 1168 cm-¹ which is the C-O stretch of the ester group reinforces this finding [74], because the C=O and C-O band pairs are typical of the ester group structure resulting from transesterification reactions [75].

Furthermore, the bands at 1,452 - 1,462 cm^{-1} were identified as bending CH_2 [76] and the weak band at 723

cm^{-1} as rocking CH_2 [77], which collectively indicate the presence of long hydrocarbon chains as part of the biodiesel chemical structure [55,79]. Overall, the existence of several components such alcohols, phenolics, alkenes, aromatics, and carboxylic acids is confirmed by the FTIR analysis of biodiesel. The FTIR spectral patterns of the 2 biodiesel samples showed significant similarities in chemical structure, indicating that both *Spirulina* sp. and *Nannochloropsis oculata* have potential as quality-equivalent biodiesel feedstock sources. Minor differences in peak position and intensity can be attributed to variations in the fatty acid composition and purity of the final product of each microalgal species. Thus, FTIR analysis provides strong evidence of the successful conversion of microalgal lipids to methyl esters, and supports the use of microalgae as a renewable feedstock for biodiesel production [54,55].

Table 2 Comparing the FTIR wavelengths of biodiesel derived from *Spirulina sp.* and *Nannochloropsis oculate.*

Wavenumber (cm^{-1})			Band assignments	Associated compound
[54], [55]	*Spirulina* sp.	*Nannochloropsis oculata*		
3346	3,415 (br, m)	3,415 (br, m)	O–H (stretching)	Hydroxyl group (alcohols, phenols)
2,800 - 3,000	2,923 (s)	2,925 (s)	C–H (asymmetric stretching, CH_2)	Aliphatic chains (alkanes)
2,800 - 3,000	2,853 (s)	2,854 (s)	C–H (symmetric stretching, CH_2)	Aliphatic chains (alkanes)
1,159 - 1,764	1,743 (vs)	1,743 (vs)	C=O (carbonyl stretch, ester)	Methyl ester (Ester carbonyl functional group in FAME)
1,159 - 1,764	1,168 (s)	1,168 (s)	C–O stretching (ester)	Methyl ester (Ester carbonyl functional group in FAME)
1,375 - 1,464	1,462 (m)	1,456 (m)	CH_2 bending	Long-chain hydrocarbons
723 - 724	723 (w)	723 (w)	CH_2 rocking	Long-chain hydrocarbons

*Abbreviations: vw, very weak, w, weak; m, medium; s, strong; vs, very strong; sh, shoulder; br, broad

Identification of FAME and fatty acid using GC-MS

The biodiesel profiles of *Spirulina* sp. and *Nannochloropsis oculata* were characterized using Gas Chromatography-Mass Spectrometry (GC-MS), with a focus on identifying the dominant fatty acid methyl esters (FAMEs) and free fatty acid compounds relevant to fuel performance. The percentage distribution of FAMEs and fatty acid content in microalgae biomass grown in culture media of tofu wastewater are shown in

Table 3. The results revealed distinct compositional patterns between the 2 microalgae species, with implications for biodiesel quality, combustion behaviour, and storage stability. For *Spirulina* sp., 3 major FAMEs were detected: methyl oleate ($C_{19}H_{36}O_2$), methyl palmitate ($C_{17}H_{34}O_2$), and methyl docosapentaenoate ($C_{23}H_{36}O_2$), with relative abundances of 0.46%, 0.40% and 0.29%, respectively. Methyl oleate, a monounsaturated FAME, was the most abundant component. This monounsaturated compound is widely recognized for its balanced fuel characteristics, favorable cold flow properties and improved oxidative stability relative to polyunsaturated FAMEs.

Nannochloropsis oculata biodiesel exhibited a markedly different profile, dominated by methyl hydroxystearate ($C_{19}H_{38}O_3$, 1.82%) and methyl palmitate (0.48%). The presence of methyl hydroxystearate, a saturated hydroxylated ester, is notable because of its potential to enhance fuel lubricity, although it may pose challenges in terms of cold flow behavior and long-term oxidative stability. The comparatively higher abundance of saturated FAMEs in

Nannochloropsis oculata suggests a biodiesel product with improved ignition quality but potentially higher cloud and pour points.

The total lipid content of *Spirulina* sp. and *Nannochloropsis oculata* in 20% tofu wastewater were 2.44% and 1.21%, respectively [36]. Because the culture conditions were the same for both species, the variations in lipid composition can be ascribed to species-specific characteristics and the algal cultivation medium. However, another *Spirulina* sp. and *Nannochloropsis oculata* strain grown in 15% Palm Oil Mill Effluent (POME) wastewater for 14 days had a total lipid content of 0.87% and 1.11%, respectively [42]. GC-MS analysis showed that the predominant fatty acid type and its composition varied depending on the type of microalgae biomassa: arachidic acid, gondoic acid, behenic acid and erucic acid. Monounsaturated Fatty Acids (MUFAs) such as arucic and gondoic acids were found in 19.81% - 29.02% and 0.77% - 0.87% of *Spirulina* sp. and *Nannochloropsis oculata,* respectively. MUFAs are the best components of biodiesel because of their oxidative stability and low thermal fluidity [79].

Table 3 (a) Composition of FAME in biodiesel from *Spirulina* sp. and *Nannochloropsis oculata* microalgae; b) Compound of fatty acid in biodiesel from *Spirulina* sp. and *Nannochloropsis oculata* microalgae

(a)

Molecular Formula	Identified Compounds	Molecular Weight	% Abundance	Lipid Type
***Spirulina* sp.**				
$C_{17}H_{34}O_2$	Methyl palmitate	270	0.40	SFA
$C_{23}H_{36}O_2$	Methyl docosapentaenoate	344	0.29	PUFA
$C_{19}H_{36}O_2$	Methyl oleate	296	0.46	MUFA
Nannochloropsis oculata				
$C_{17}H_{34}O_2$	Methyl palmitate	270	0.48	SFA
$C_{19}H_{38}O_3$	Methyl hydroxystearate	314	1.82	SFA

(b)

Molecular Formula	Identified Compounds	Molecular Weight	% Abundance	Lipid Type
***Spirulina* sp.**				
$C_{20}H_{38}O_2$	Arachidic acid	310	0.32	SFA
$C_{20}H_{40}O_2$	Gondoic acid	312	0.77	MUFA
$C_{22}H_{42}O_2$	Erucic acid	338	29.02	MUFA
Nannochloropsis oculata				
$C_{20}H_{38}O_2$	Arachidic acid	310	0.55	SFA
$C_{20}H_{40}O_2$	Gondoic acid	312	0.87	MUFA
$C_{22}H_{42}O_2$	Behenic acid	340	1.81	SFA
$C_{22}H_{44}O_2$	Erucic acid	338	19.81	MUFA

Previous study

Table 4 presents a comparative analysis of the biodiesel yield from various microalgae species through *in situ* transesterification. A key observation is the wide variation in biodiesel yield, ranging from 9% [80] to 98% [81]. This disparity underscores the critical role of species selection, biomass conditions, catalyst type, and reaction parameters. Notably, *Chlorella vulgaris* exhibited high yields in several studies, with 77.64% [82] under dry conditions using KOH and 83.4% [1] using H_2SO_3. Similarly, *Rhodotorula glutinis* achieved 80% yield under an extended reaction time (600 min) with H_2SO_3 [83] indicating the effectiveness of acid catalysts and longer reaction times for certain species. These results highlight that not only the algal species but

also pre-treatment and transesterification conditions significantly affect biodiesel productivity.

Compared with these studies, the present research demonstrates promising but moderate biodiesel yields from *Spirulina* sp. and *Nannochloropsis oculata* using wet biomass and KOH as a catalyst under relatively short reaction times (120 min) and a moderate temperature of 80 °C. The highest yield obtained was 17.5% for *Nannochloropsis oculata*, which was lower than the yields reported for dried biomass over extended durations (e.g., 98% by *Chlorella vulgaris* in Malekghasemi [83] or 80% by *Rhodotorula glutinis*. Furthermore, the yield achieved surpasses several other wet biomass studies, such as Musa [80]**,** who reported only 9–11% yields for *Nannochloropsis oculata*. This suggests that this process has great promise for producing useful biodiesel from wet algal biomass with additional tuning, especially with regard to catalyst choices and the methanol-to-biomass ratio.

In addition, biomass condition (wet vs. dried) emerged as a critical factor influencing transesterification efficiency. Dried biomass generally produces higher yields owing due to reduced moisture interference, which otherwise impedes catalyst activity. For instance, *Chlorella* sp. and *Dunaliella salina* yielded 72.8% [84] under dry conditions, whereas *Nannochloropsis oculata* processed wet under H_2SO_3 only yielded 11% [80]. The choice of solvent also plays a vital role; methanol remains the most commonly used solvent because of its polarity and cost-effectiveness. However, the combination of methanol with palm oil [85] or ionic liquids [81] significantly enhanced the biodiesel yield. The latter achieved the highest yield (98%), emphasizing the potential of hybrid solvent systems to improve the lipid extraction and conversion efficiency [81].

Table 4 Comparison study of biodiesel yield from microalgae via *in situ* transesterification.

Microalgae	Cultivation medium	Biomass condition	Catalyst	Solvent	Reaction condition			Biodiesel yield (%)	Previous findings
					Solvent molar: Biomass ratio (m/v)	Temp (°C)	Time (min)		
Spirulina sp.	Tofu liquid waste	Wet	KOH	Methanol	7:1	80	120	12.5	This study
Nannochloropsis oculata	Tofu liquid waste	Wet	KOH	Methanol	7:1	80	120	17.5	This study
Chlorella sp.	Organic fertilizer	Dried	H_2SO_4	Methanol	7:1	65	480	48	[86]
Chlorella minutissima	BG-11	Dried	NaOH	n-hexane and Methanol	NA	65	240	72.8	[84]
Chaetoceros calcitrans	Seawater	Dried	H_2SO_4	n-hexane and ethanol	1:40	60	60	21.59	[87]
Nannochloropsis sp.	NA	Wet	KOH	n-Hexane and Methanol	NA	65	960	35	[88]
Rhodotorula glutinis	BCRC 22360	Wet	H_2SO_4	Methanol	100:1	90	600	80	[83]
Tetradesmus obliquus	BG11	Wet	NaOH	n-Hexane	NA	90	60	26.5	[89]
Tetradesmus obliquus	BG11	Wet	H_2SO_4	n-Hexane	NA	90	60	25.7	
Chlorella vulgaris	NA	Dried	KOH	Methanol	1:12	NA	40	77.64	[82]

Microalgae	Cultivation medium	Biomass condition	Catalyst	Solvent	Reaction condition			Biodiesel yield (%)	Previous findings
					Solvent molar: Biomass ratio (m/v)	Temp (°C)	Time (min)		
Nannochloropsis oculata *Dunaliella salina*	F/2-Si	Wet	H_2SO_4	Methanol	1:600	60	60	9 11	[80]
Spirulina sp.	NA	Dried	KOH	Methanol and palm oil	5:1	60	120	16.69	[85]
Chlorella vulgaris	Tris-acetate-phosphate (TAP) medium	Dried	KOH	Methanol and ionic liquid	8:1	102.4	276	98	[81]
Chlorella sp. *Tetraselmis* sp.	Artificial wastewater (phenols)	Dried	CaO/Al_2O_3	Methanol	12:1	45	240	30.4	[90]
Arthrospira Spirulina platensis	NA	Dried	H_2SO_4	Methanol	9:1	NA	30	83.4	[1]
Chlorella vulgaris	NA	Wet	H_2SO_4	Methanol, n-butanol, tetrahydro-furan	NA	60	120	33	[91]
Scenedesmus sp.	Organic fertilizer	Dried	Immobilized lipase	Methanol	8:1	35	360	19.3	[92]

Similarly, reaction conditions such as temperature, time, and catalyst type are critical for optimizing the biodiesel yield. Most studies report favorable outcomes in the range of 60 - 90 °C, with extended times (e.g., 480 - 600 min) enhancing the yield, particularly with acid catalysts. *Chlorella* sp. produced a 48% yield after 480 min using H_2SO_3 [86], while *Rhodotorula glutinis* achieved an 80% yield after 600 min [83]. Catalysts significantly influence reaction kinetics; acid catalysts such as H_2SO_3 are effective in both esterification and transesterification, especially for microalgae with a high free fatty acid (FFA) content. Base catalysts (KOH and NaOH) tend to be faster but are sensitive to FFAs and moisture, which can reduce their efficiency [92]. Interestingly, the immobilized lipase used in *Scenedesmus* sp. [92] offers a greener alternative, albeit with a lower yield (19.3%), suggesting the potential for further optimization of enzymatic processes. Overall, Error! Reference source not found. shows that optimal biodiesel production from microalgae depends on multiple interconnected factors: species selection, biomass drying, solvent system, and catalyst choice.

Conclusions

This study verified that using in-situ transesterification to produce biodiesel from *Spirulina* sp. and *Nannochloropsis oculata* grown in tofu wastewater is feasible. Using methanol and a KOH catalyst, process optimization showed that the highest biodiesel yields 12.5% for *Spirulina* sp. and 17.5% for *Nannochloropsis oculata* were obtained at a methanol-to-biomass ratio of 7:1 with a reaction time of 120 min. These results demonstrate the significant effect of reaction parameters on transesterification efficiency and highlight the potential of tofu wastewater as a sustainable medium for microalgal biodiesel production. FTIR analysis confirmed the formation of methyl esters by detecting key functional groups, such as C=O and C–O. GC-MS further validated the biodiesel composition and identified important FAMEs, such as methyl palmitate and methyl oleate. The utilization of tofu wastewater further underscores the dual benefit of supporting microalgal growth while promoting industrial waste valorization. Although these findings

highlight the promise of this approach, they remain preliminary. Further studies are required to evaluate physicochemical fuel properties and performance parameters, as well as to explore strategies for lipid enhancement, catalyst optimization, and large-scale process assessment through techno-economic and life cycle analyses.

Acknowledgements

The authors gratefully acknowledge the Research and Community Service Institution, Institut Teknologi Kalimantan, Indonesia, for providing financial support for this project through the Research Grant (Contract Number: 13369/IT10.L1/PPM.04/2025).

Declaration of generative AI in scientific writing

In developing this manuscript, the authors utilized ChatGPT 4.0 to improve the English language and assist with proofreading. Following its use, the authors conducted a detailed review and made all necessary adjustments, fully accountable for the final content published.

CRediT author statement

Nita Ariestiana Putri: Conceptualization, Methodology, Supervision, Validation, Writing-original draft; **Resti Nurmala Dewi**: Methodology, Formal analysis, Validation, Writing-original draft; **Benny Arif Pambudiarto:** Formal analysis; Validation Software; **Jefri Pandu Hidayat:** Validation, writing - review & editing; **Nie Luhh Dhian Forestry:** Investigation, Formal analysis, Visualization; **Alvin Nur Rahman:** Investigation; Formal analysis, Visualization; **Rizka Lestari:** Validation**;** Formal analysis; Writing - review & editing; **Muhamad Maulana Azimatun Nur:** Validation, Writing - review & editing; **Ira Nurhayati Djarot:** Supervision; Review Final Paper; **Asful Hariyadi:** Review & editing, **Elizabeth Desfelia Ciciolini Sidabutar:** Writing - review & editing.

References

[1] AK Koech, A Kumar and ZO Siagi. *In Situ* transesterification of *spirulina* microalgae to produce biodiesel using microwave irradiation. *Journal of Energy* 2020; **2020**, 1-10.

[2] A Rahman, R Richards, P Dargusch and D Wadley. Pathways to reduce Indonesia's dependence on oil and achieve longer-term decarbonization. *Renewable Energy* 2023; **202(7830)**, 1305-1323.

[3] KS Kumar, S Alqarni, S Islam and MA Shah. Royal poinciana biodiesel blends with 1-butanol as a potential alternative fuel for unmodified research engines. *ACS Omega* 2024; **9(12)**, 13960-13974.

[4] D Neupane. Biofuels from renewable sources, a potential option for biodiesel production. *Bioengineering* 2023; **10(1)**, 29.

[5] Monika, S Banga and VV Pathak. Biodiesel production from waste cooking oil: A comprehensive review on the application of heterogenous catalysts. *Energy Nexus* 2023; **10**, 100209.

[6] T Issariyakul and AK Dalai. Biodiesel from vegetable oils. *Renewable and Sustainable Energy Reviews* 2014; **31**, 446-471.

[7] P Andreo-Martínez, VM Ortiz-Martínez, MJ Salar-García, JM Veiga-del-Baño, A Chica and J Quesada-Medina. Waste animal fats as feedstock for biodiesel production using non-catalytic supercritical alcohol transesterification: A perspective by the PRISMA methodology. *Energy for Sustainable Development* 2022; **69(1)**, 150-163.

[8] S Pandey, I Narayanan, R Selvaraj, T Varadavenkatesan and R Vinayagam. Biodiesel production from microalgae: A comprehensive review on influential factors, transesterification processes, and challenges. *Fuel* 2024; **367**, 131547.

[9] SK Hoekman, A Broch, C Robbins, E Ceniceros and M Natarajan. Review of biodiesel composition, properties, and specifications. *Renewable and Sustainable Energy Reviews* 2012; **16(1)**, 143-169.

[10] AA Babadi, S Rahmati, R Fakhlaei, B Barati, S Wang, W Doherty and KK Ostrikov. Emerging technologies for biodiesel production: Processes, challenges, and opportunities. *Biomass and Bioenergy* 2022; **163**, 106521.

[11] K Gaurav, K Neeti and R Singh. Microalgae-based biodiesel production and its challenges and future

opportunities: A review. *Green Technologies and Sustainability* 2024; **2(1)**, 100060.

[12] V Mittal and UK Ghosh. Optimization of biodiesel production from *Spirulina microalgae* via nanocatalytic transesterification process. *Bioresource Technology Reports* 2023; **23**, 101504.

[13] MA Davoodbasha, A Pugazhendhi, JW Kim, SY Lee and T Nooruddin. Biodiesel production through transesterification of Chlorella vulgaris: Synthesis and characterization of CaO nanocatalyst. *Fuel* 2021; **300**, 1-8.

[14] YS Pradana, RN Dewi, KD Livia, F Arisa, RB Cahyono and A Budiman. Advancing biodiesel production from microalgae *Spirulina* sp. By a simultaneous extraction-transesterification process using palm oil as a co-solvent of methanol. *Open Chemistry* 2020; **18(1)**, 833-842.

[15] JVA Raj, B Bharathiraja, B Vijayakumar, S Arokiyaraj, J Iyyappan and RP Kumar. Biodiesel production from microalgae *Nannochloropsis oculata* using heterogeneous poly ethylene glycol (PEG) encapsulated $ZnOMn^{2+}$ nanocatalyst. *Bioresource Technology* 2019; **282(5)**, 348-352.

[16] M Mahfud, U Kalsum and V Aswie. Biodiesel production through catalytic microwave in-situ transesterification of microalgae (Chlorella sp.). *International Journal of Renewable Energy Development* 2020; **9(1)**, 113-117.

[17] YW Mirzayanti, L Marlinda, H Irawan, MA Muttaqii, Z Ma'sum, NP Asri and JM Chern. Performance of *in-situ* stirring batch reactor transesterification of nannochloropsis sp microalgae into biodiesel. *International Journal of Technology* 2024; **15(4)**, 859-869.

[18] C Sanjurjo, P Oulego, M Bartolomé, E Rodríguez, R Gonzalez and AH Battez. Biodiesel production from the microalgae *Nannochloropsis gaditana*: Optimization of the transesterification reaction and physicochemical characterization. *Biomass and Bioenergy* 2024; **185(9)**, 107240.

[19] YW Mirzayanti, E Ningsih, AC Ni'am, M Al-Muttaqii, A Awaludin, IM Sukono, IN Noorsy, NP Asri, JM Chern, and Z Ma'sum. Enhanced biofuel production from Nannochloropsis sp. via microwave-assisted extraction in-Situ transesterification using CaO/hydrotalcite catalyst. *Case Studies in Chemical and Environmental Engineering* 2025; **11**, 101234.

[20] P Purkan, A Safa, A Abdulloh, A Baktir, R Arissirajudin, H Hermansyah and SW Kim. *In-situ* biodiesel synthesis from microalgae *Nannochloropsis oculata* using nio nanocatalyst. *RASAYAN Journal of Chemistry* 2021; **14(3)**, 2097-2103.

[21] A Rahman, N B Prihantini and Nasruddin. Fatty acid of microalgae as a potential feedstock for biodiesel production in Indonesia. *AIP Conference Proceedings* 2019; **2062(1)**, 020059.

[22] A Bora, A S Thondi Rajan, K Ponnuchamy, G Muthusamy and A Alagarsamy. Microalgae to bioenergy production: Recent advances, influencing parameters, utilization of wastewater - a critical review. *Science of The Total Environment* 2024; **946(1)**, 174230.

[23] N Politaeva, I Ilin, K Velmozhina and P Shinkevich. Carbon dioxide utilization using chlorella microalgae. *Environments* 2023; **10(7)**, 109.

[24] SA Razzak, K Bahar, KMO Islam, AK Haniffa, MO Faruque, SMZ Hossain and MM Hossain. Microalgae cultivation in photobioreactors: sustainable solutions for a greener future. *Green Chemical Engineering* 2024; **5(4)**, 418-439.

[25] RN Dewi, MMA Nur, RP Astuti, W Andriyanto, FCA Panjaitan, D Febrianti, IGA Budiadnyani, SPSD Utari, PN Samanta and ML Perceka. Bioremediation of seafood processing wastewater by microalgae: Nutrient removal, and biomass, lipid and protein enhancement. *Environmental Engineering Research* 2024; **29(6)**, 230673.

[26] SF Mohsenpour, S Hennige, N Willoughby, A Adeloye and T Gutierrez. Integrating micro-algae into wastewater treatment: A review. *Science of The Total Environment* 2021; **752**, 142168.

[27] H Feng, L Jin, Y Chen, J Ji, Z Gong, W Hu, C Ying, Y Liang and J Li. Tofu wastewater as a carbon source flowing into municipal wastewater treatment plants for reductions of costs and greenhouse gas emissions. *Journal of Environmental Management* 2024; **370**, 122550.

[28] SK Wang, X Wang, J Miao and YT Tian. Tofu whey wastewater is a promising basal medium for

microalgae culture. *Bioresource Technology* 2018; **253**, 79-84.

[29] S Elystia, AS Lestari and SR Muria. Increase in lipid content and biomass of scenedesmus sp. microalgae from tofu wastewater cultivation media as raw material for biodiesel. *Jurnal Tek Lingkungan* 2019; **5(2)**, 19-28.

[30] RN Dewi, NA Putri, Hadiyanto and MMA Nur. Enhancement of lipid production from botryococcus braunii growing on tofu wastewater by employing mixotrophic cultivation condition. *Waste and Biomass Valorization* 2025; **16(7)**, 3575-3589.

[31] Dianursanti, BT Rizkytata, MT Gumelar and TH Abdullah. Industrial tofu wastewater as a cultivation medium of microalgae *Chlorella vulgaris. Energy Procedia* 2014; **47**, 56-61.

[32] M Christwardana and H Hadiyanto. Cultivating microalgae botryococcus braunii in tofu whey medium. *Journal of Bioresources and Environmental Sciences* 2022; **1(1)**, 12-19.

[33] H El Bakraoui, A Malaki, M Slaoui and C Laroche. *Chromochloris zofingiensis*-based treatment of whey wastewater for biorefinery application: Biomass, nutrient removal, astaxanthin and lipid production. *Applied Sciences* 2025; **15(11)**, 5832.

[34] R Rusydi and A Yakupitiyage. Potential of tofu wastewater as a medium in Cyanobacteria Nostocmuscorum culture for its biomass and lipid production. *IOP Conference Series: Earth and Environmental Science* 2019; **348(1)**, 012087.

[35] C Putnarubun, M Rettob, IBW Gunam and SK Balubun. Utilization of tofu waste as a cultivation media for tetraselmis sp microalgae in preliminary study of biodiesel production. *International Journal of Basic & Applied Sciences* 2015; **04(15)**, 13-16.

[36] NA Putri, RN Dewi, RA Yuniar and RA Mauliasantri. Transforming tofu waste into a growth medium: Boosting biomass and proximate content of microalgae. *International Journal of Marine Engineering Innovation and Research* 2024; **9(4)**, 776-786.

[37] MP Spínola, AR Mendes and JAM Prates. Chemical composition, bioactivities, and applications of spirulina (*Limnospira platensis*) in food, feed, and medicine. *Foods* 2024; **13(22)**, 3656.

[38] B Marjanović, M Benković, T Jurina, TS Cvetnić, D Valinger, JG Kljusurić and AJ Tušek. Bioactive compounds from Spirulina spp.-nutritional value, extraction, and application in food industry. *Separations* 2024; **11(9)**, 257.

[39] C Taiti, G Stefano, E Percaccio, SD Giacomo, M Iannone, A Marianelli, AD Sotto and S Garzoli. Addition of spirulina to craft beer: Evaluation of the effects on volatile flavor profile and cytoprotective properties. *Antioxidants* 2023; **12(5)**, 1021.

[40] A Prasetyo, I Khaerunnisa, SD Volkandari, AA Yano, F Shidiq, B Haryanto, NA Fitrianto, C Hanim and S Sugiharto. Composition and essential nutrients of cyanobacterium meal produced from spirulina (*Arthrospira platensis*) fermented by yeast on small-scale. *Case Studies in Chemical and Environmental Engineering* 2024; **10**, 100940.

[41] ES Sofiyah and IWK Suryawan. Cultivation of Spirulina platensis and *Nannochloropsis oculata* for nutrient removal from municipal wastewater. *Rekayasa* 2021; **14(1)**, 93-97.

[42] NA Putri, RN Dewi, R Lestari, RA Yuniar, LM Ma'arif and R Erianto. Microalgae as a bioremediation agent for palm oil mill effluent: Production of biomass and high added value compounds. *Jurnal Rekayasa Kimia & Lingkungan* 2023; **18(2)**, 149-161.

[43] S Banerjee, WE Hew, H Khatoon, M Shariff and FM Yusoff. Growth and proximate composition of tropical marine Chaetoceros calcitrans and *Nannochloropsis oculata* cultured outdoors and under laboratory conditions. *African Journal of Biotechnology* 2011; **10(8)**, 1375-1383.

[44] X Liu, D Yu, H Luo and C Li. Green solvents for lipid extraction from microalgae to produce biodiesel. *Frontiers in Chemistry* 2022; **10**, 884274.

[45] S K Bhatia, SK Bhatia, RK Bhatia, JM Jeon, A Pugazhendhi, MK Awasthi, D Kumar, G Kumar, JJ Yoon and YH Yang. An overview on advancements in biobased transesterification methods for biodiesel production: Oil resources, extraction, biocatalysts, and process intensification technologies. *Fuel* 2021; **285**, 119117.

[46] F Ideris, MF Muaz, AH Shamsuddin, NM Saifuddin, F Kusumo, IMR Fattah and TMI Mahlia. Progress on conventional and advanced techniques of *in situ* transesterification of microalgae lipids for biodiesel production. *Energies* 2022; **15(19)**, 7190.

[47] NT Thanh, M Mostapha, MK Lam, S Ishak, YK Dasan, JW Lim, IS Tan, SY Lau, BLF Chin and T Hadibarata. Fundamental understanding of *in-situ* transesterification of microalgae biomass to biodiesel: A critical review. *Energy Conversion and Management* 2022; **270**, 116212.

[48] API Cercado, FC Ballesteros and SC Capareda. Biodiesel from three microalgae transesterification processes using different homogenous catalysts. *International Journal of Technology* 2018; **9(4)**, 645-651.

[49] G Ma, W Hu, H Pei, L Jiang, Y Ji and R Mu. Study of KOH/Al_2O_3 as heterogeneous catalyst for biodiesel production via *in situ* transesterification from microalgae. *Environmental Technology* 2015; **36(5-8)**, 622-627.

[50] S Dalvi and S Sonawane. Biodiesel (fatty acid methyl ester) from Chlorococcalean Chlorella vulgaris by single step in-situ transesterification. *Indian Journal of Science and Technology* 2022; **15(12)**, 535-541.

[51] RN Dewi, Mahreni, MMA Nur, AA Siahaan and AC Ardhi. Enhancing the biomass production of microalgae by mixotrophic cultivation using virgin coconut oil mill effluent. *Environmental Engineering Research* 2023; **28(2)**, 0-2.

[52] SS Mahmood and AM Al-yaqoobi. Production of biodiesel by using CaO nano-catalyst synthesis from mango leaves extraction. *International Journal of Renewable Energy Development* 2024; **13(6)**, 1025-1034.

[53] MR Talaghat, S Mokhtari and M Saadat. Modeling and optimization of biodiesel production from microalgae in a batch reactor. *Fuel* 2020; **280(10)**, 118578.

[54] H Kamyab, S Chelliapan, MF Md Din, CT Lee, S Rezania, T Khademi and CPC Bong. Isolate new microalgal strain for biodiesel production and using FTIR spectroscopy for assessment of pollutant removal from palm oil mill effluent (POME). *Chemical Engineering Transactions* 2018; **63**, 91-96.

[55] S Boro, B Das, S Brahma, B Basumatary, SF Basumatary and S Basumatary. Biodiesel production using areca nut (*Areca catechu* L.) leaf ash-K_2CO_3 catalyst via transesterification from an oil blend of three different feedstocks. *Sustainable Chemistry for the Environment* 2024; **8(1)**, 100164.

[56] D Surendhiran, M Vijay and AR Sirajunnisa. Biodiesel production from marine microalga *Chlorella salina* using whole cell yeast immobilized on sugarcane bagasse. *Journal of Environmental Chemical Engineering* 2014; **2(3)**, 1294-1300.

[57] SB Masango, PT Ngema, OA Olagunju and S Ramsuroop. The effect of reaction temperature, catalyst concentration and alcohol ratio in the production of biodiesel from raw and purified castor oil. *Advances in Chemical Engineering and Science* 2024; **14(03)**, 137-154.

[58] IA Musa. The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process. *Egyptian Journal of Petroleum* 2016; **25(1)**, 21-31.

[59] M Kamel, L Vitali, A Abdelnour, AA Abdullah and AN Hassan. Supercritical methanolysis of leather tanning waste biomass: experimental investigation, process simulation, and sustainable biodiesel production. *Beni-Suef University Journal of Basic and Applied Sciences* 2025; **14(1)**, 47.

[60] IW Sudana, Helmiyati and RT Yunarti. Alginate-CMC/Fe_3O_4-CaO nanocomposite as a catalyst for synthesis of biodiesel from waste cooking oil. *IOP Conference Series Earth and Environmental Science* 2021; **846(1)**, 012008.

[61] V Kumar, M Muthuraj, B Palabhanvi, AK Ghoshal and D Das. Evaluation and optimization of two stage sequential *in situ* transesterification process for fatty acid methyl ester quantification from microalgae. *Renewable Energy* 2014; **68**, 560-569.

[62] RJ Sánchez, MB Fernández and SM Nolasco. Ethanol extraction of canola oil: Kinetics and effects of type of solvent and microwave-pretreatment. *Oilseeds Fats Crop Lipids* 2019; **26(9)**, 27.

[63] N Martínez, N Callejas, EG Morais, JAV Costa, I Jachmanián and I Vieitez. Obtaining biodiesel from microalgae oil using ultrasound-assisted in-situ alkaline transesterification. *Fuel* 2017; **202**, 512-519.

[64] SH Shuit, K T Lee, AH Kamaruddin and S Yusup. Reactive extraction and *in situ* esterification of *Jatropha curcas* L. seeds for the production of biodiesel. *Fuel* 2010; **89(2)**, 527-530.

[65] JS Lemões, RCMA Sobrinho, SP Farias, RRD Moura, EG Primel, PC Abreu, AF Martins and MGM D'Oca. Sustainable production of biodiesel from microalgae by direct transesterification. *Sustainable Chemistry and Pharmacy* 2016; **3**, 33-38.

[66] A Sathish, BR Smith and RC Sims. Effect of moisture on *in situ* transesterification of microalgae for biodiesel production. *Journal of Chemical Technology and Biotechnology* 2014; **89(1)**, 137-142.

[67] H Cao, Z Zhang, X Wu and X Miao. Direct biodiesel production from wet microalgae biomass of Chlorella pyrenoidosa through *in situ* transesterification. *BioMed Research International* 2013; **2013**, 930686.

[68] BD Wahlen, RM Willis and LC Seefeldt. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology* 2011; **102(3)**, 2724-2730.

[69] AW Go, S Sutanto, LK Ong, PL Tran-Nguyen, S Ismadji and YH Ju. Developments *in in-situ (trans)* esterification for biodiesel production: A critical review. *Renewable and Sustainable Energy Reviews* 2016; **60**, 284-305.

[70] AS Elgharbawy, WA Sadik, OM Sadek and MA Kasaby. A review on biodiesel feedstocks and production technologies. *Journal of the Chilean Chemical Society* 2021; **66(1)**, 5098-5109.

[71] M Arif, Y Li, MM El-Dalatony, C Zhang, X Li and ES Salama. A complete characterization of microalgal biomass through FTIR/TGA/CHNS analysis: An approach for biofuel generation and nutrients removal. *Renewable Energy* 2021; **163(1)**, 1973-1982.

[72] Sukarni, Sudjito, N Hamidi, U Yanuhar and ING Wardana. Potential and properties of marine microalgae *Nannochloropsis oculata* as biomass fuel feedstock. *International Journal of Energy and Environmental Engineering* 2014; **5(4)**, 279-290.

[73] A Matwijczuk, G Zając, R Kowalski, M Kachel-Jakubowska and M Gagoś. Spectroscopic studies of the quality of fatty acid methyl esters derived from waste cooking oil. *Polish Journal of Environmental Studies* 2017; **26(6)**, 2643-2650.

[74] B Hazmi, U Rashid, S Kawi, WNAW Mokhtar, TCS Yaw, BR Moser and A Alsalme. Palm fatty acid distillate esterification using synthesized heterogeneous sulfonated carbon catalyst from plastic waste: Characterization, catalytic efficacy and stability, and fuel properties. *Process Safety and Environmental Protection* 2022; **162**, 1139-1151.

[75] S Verma, D Sahu and BO Almutairi. Production and characterization of biodiesel fuel produced from third-generation feedstock. *Frontiers in Materials* 2024; **11**, 1-9.

[76] HT Ahmed and OG Abdullah. Structural and ionic conductivity characterization of PEO:MC-NH4I proton-conducting polymer blend electrolytes based films. *Results in Physics* 2020; **16**, 102861.

[77] KR Rodriguez, H Tian, JM Heer, S Teeters-Kennedy and JV Coe. Interaction of an infrared surface plasmon with an excited molecular vibration. *The Journal of Chemical Physics* 2007; **126(15)**, 151101.

[78] OA Falowo, MI Oloko-Oba and E Betiku. Biodiesel production intensification via microwave irradiation-assisted transesterification of oil blend using nanoparticles from elephant-ear tree pod husk as a base heterogeneous catalyst. *Chemical Engineering and Processing - Process Intensification* 2019; **140**, 157-170.

[79] Y Cao, W Liu, X Xu, H Zhang, J Wang and M Xian. Production of free monounsaturated fatty acids by metabolically engineered Escherichia coli. *Biotechnology for Biofuels* 2014; **7(1)**, 1-11.

[80] SD Musa. 2020, Process intensification of biodiesel production from algae using foam flotation column. Thesis. Newcastle University, Newcastle, England.

[81] S Malekghasemi, HR Kariminia, NK Plechkova and VCA Ward. Direct transesterification of wet

microalgae to biodiesel using phosphonium carboxylate ionic liquid catalysts. *Biomass and Bioenergy* 2021; **150**, 106126.

[82] R Adhiputra, M Utami, EA Suyono, A Budiman, PL Hariani, AS Pratiwi and K Wijaya. Simultaneous extraction and *in-situ* transesterification of *chlorella vulgaris* using microwave-assisted method for biodiesel production. *Korean Journal of Materials Research* 2021; **31(4)**, 181-187.

[83] SJ Chen, IC Kuan, YF Tu, SL Lee and CY Yu. Surfactant-assisted *in situ* transesterification of wet Rhodotorula glutinis biomass. *Journal of Bioscience and Bioengineering* 2020; **130(4)**, 397-401.

[84] R Arora, KS Nigha, P Verma, RK Wanchoo and AP Toor. Microalgal synthesis of the biodiesel employing simultaneous extraction and esterification via heterogeneous catalyst. *Journal of the Indian Chemical Society* 2024; **101(2)**, 101123.

[85] YS Pradana, RN Dewi, KD Livia and F Arisa. Advancing biodiesel production from microalgae *Spirulina* sp. by a simultaneous extraction–transesterification process using palm oil as a co-solvent of methanol. *Open Chemistry* 2020; **18(1)**, 833-842.

[86] I Telussa, EG Fransina and K Kalauw. Synthesis of methyl ester from microalgae chlorella sp. TAD using the *in-situ* transesterification method. *Jurnal Teknik Kimia dan Lingkungan* 2024; **8(1)**, 63-69.

[87] R Shomal, H Hisham, A Mlhem, R Hassan and S Al-Zuhair. Simultaneous extraction-reaction process for biodiesel production from microalgae. *Energy Reports* 2019; **5**, 37-40.

[88] P Purkan, H Nurlaila, A Baktir, S Hadi and W Soemarjati. Microalgal lipid from Chaetoceros calcitrans and its conversion to biodiesel through ex and in-situ transesterification. *Rasayan Journal of Chemistry* 2022, 43-52.

[89] F Baqi, RSI Putri and YW Mirzayanti. Proses pembuatan biodiesel dari mikroalga Nannochloropsis sp. menggunakan metode transesterifikasi in-situ dengan katalis KOH. *Equilibrium Journal of Chemical Engineering* 2022; **6(2)**, 92.

[90] N Singh, K Kumar, A Goyal and V S Moholkar. Ultrasound-assisted biodiesel synthesis by in–situ transesterification of microalgal biomass: Optimization and kinetic analysis. *Algal Research* 2022; **61**, 102582.

[91] M Abujayyab and AZ Sulaiman. Use of microalgae for simultaneous industrial wastewater treatment and biodiesel production. *International Journal of Environmental Research* 2020; **14(3)**, 311-322.

[92] T T Nguyen, MK Lam, Y Uemura, N Mansor, JW Lim, PL Show, IS Tan and S Lim. High biodiesel yield from wet microalgae paste via *in-situ* transesterification: Effect of reaction parameters towards the selectivity of fatty acid esters. *Fuel* 2020; **272**, 117718.

[93] R Shomal, H Hisham, A Mlhem, R Hassan and S Al-Zuhair. Simultaneous extraction-reaction process for biodiesel production from microalgae. *Energy Reports* 2019; **5**, 37-40.