

Nonlinear enhancement of in-situ transesterification in marine diatoms under microwave irradiation

Heni Anggorowati¹, Tunjung Wahyu Widayati¹, Geza Satya Danera¹, Mawari Gita Wahyuni¹, Putri Restu Dewati^{1*}

¹ Department of Chemical Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta, Jl. Seturan Raya C No.405, Daerah Istimewa Yogyakarta, Indonesia

* Corresponding author's e-mail: putrirestudewati@upnyk.ac.id

ABSTRACT

The development of rapid and efficient conversion routes is essential to improve the feasibility of microalgae-based biodiesel. This study investigates the nonlinear enhancement of microwave-assisted in-situ transesterification using the marine diatom *Skeletonema costatum* as a model feedstock. A response surface methodology (RSM) based on a Box–Behnken Design was applied to evaluate the effects of microwave power (100–600 W), reaction time (2–8 min), and microalgae-to-solvent ratio (1:30–1:50 g/mL) on biodiesel yield under microwave irradiation. The results revealed a pronounced nonlinear response among process variables, with the quadratic effect of microwave power emerging as the dominant factor influencing biodiesel yield. The optimal condition yielded 96.169% biodiesel (dry biomass basis) at 600 W, 5 minutes, and a 1:50 (g/mL) ratio. GC–MS analysis confirmed a methyl ester content of 79.53%, predominantly hexadecanoic acid methyl ester (73.61%), demonstrating the suitability of marine diatoms as promising biodiesel feedstocks. Microwave irradiation significantly intensified the transesterification process by reducing reaction time while maintaining high conversion efficiency. This study is limited to laboratory-scale experiments and a defined operational window, and further work is needed to assess scalability, energy efficiency, and broader process conditions. Nevertheless, the findings provide practical insight into fast and simplified biodiesel production from marine microalgae. The main contribution of this work lies in demonstrating nonlinear process enhancement under microwave irradiation and providing a systematic framework for understanding microwave-driven in-situ transesterification in marine diatoms.

Keywords: biodiesel, in-situ transesterification, microwave-assisted extraction, *Skeletonema costatum*, response surface methodology.

INTRODUCTION

Global energy systems remain heavily dependent on fossil fuels, accounting for approximately 80% of total energy consumption, with 58% directly utilized in the transportation sector (Galusnyak et al., 2024; Mardhiah et al., 2017). This dependence contributes substantially to greenhouse gas emissions and environmental degradation (Baqi et al., 2022; Pekkoh et al., 2024). According to the World Health Organization, ambient air pollution causes more than 4.2 million premature deaths annually, with 91% of the global population exposed to air quality levels exceeding

recommended limits (Naseef and Tulaimat, 2025). These pressures highlight the urgent need for low-carbon liquid fuels compatible with existing transport infrastructure, positioning biodiesel as a strategic renewable alternative (Barnard et al., 2007; Ma and Wang, 2025).

Biodiesel feedstocks are commonly categorized into four generations based on resource origin (Sanjurjo et al., 2024). While first- and second-generation biodiesel rely on edible and non-edible crops, third-generation biodiesel derived from microalgae has attracted increasing attention due to high areal productivity and minimal competition with arable land (Zahedi et

al., 2024). Microalgae can convert CO₂ into biomass 15–300 times faster than terrestrial plants and exhibit rapid cell division rates (Narayanan, 2024; Scott et al., 2010). Beyond productivity, their lipid profiles are tunable through cultivation conditions, making them attractive platforms for controlled biofuel synthesis rather than passive biomass sources.

Biodiesel production from microalgae generally follows two principal pathways. The conventional approach involves lipid extraction followed by transesterification to produce fatty acid methyl esters (FAME) and glycerol, often requiring extended processing time, multiple solvent steps, and higher operational costs (Widyastuti and Dewi, 2015). In contrast, in-situ transesterification integrates lipid extraction and conversion into a single step, potentially reducing solvent demand and process time (Ehimen et al., 2010; Go et al., 2016). In this method, methanol and an acid catalyst directly interact with the biomass matrix (Kalsum et al., 2018). However, mass transfer limitations and incomplete cell disruption remain critical constraints affecting conversion efficiency. Co-solvents have been introduced to enhance lipid solubility and reaction kinetics (Dianursanti et al., 2015), yet systematic evaluation of interacting process parameters remains limited.

Microwave-assisted extraction (MAE) has emerged as a promising intensification strategy due to its rapid volumetric heating mechanism, resulting from direct interaction between microwave radiation and polar molecules. This dielectric heating enhances intracellular water evaporation and may induce structural disruption of cell walls (Huschek et al., 2022). Compared with conventional heating, MAE can reduce processing time and solvent consumption (Wani and Patidar, 2025). Nevertheless, it remains unclear whether microwave irradiation merely accelerates bulk thermal effects or induces synergistic dielectric and structural phenomena that enhance lipid accessibility during in-situ transesterification. In this study, the microwave system was modified to enable controlled in-situ transesterification through condenser integration and magnetic stirring.

Several studies have reported high biodiesel conversion efficiencies from freshwater microalgae species. Li et al. (2007) achieved 98.15% conversion from *Chlorella protothecoides*, while Al-Humairi et al. (2022) and Nirmala and Dawn (2021) reported yields of approximately 96%

from *Chlorella vulgaris* and *Chlorella variabilis*, respectively. However, significant variability has also been observed, such as the 46.56% FAME yield reported by Umamaheswari et al. (2020) from *Chlorella pyrenoidosa*. These discrepancies suggest that species-specific structural and compositional factors strongly influence conversion efficiency. To date, no study has systematically evaluated microwave-assisted in-situ transesterification of *Skeletonema costatum*, particularly considering nonlinear interactions among operational parameters. Among marine microalgae, *Skeletonema costatum* has demonstrated lipid contents ranging from 24.4–28.2% (Pérez et al., 2017; Rengga et al., 2019). Its fatty acid composition, dominated by palmitoleic acid (C16:1) and myristic acid (C14:1) (Katili et al., 2012), falls within the carbon chain distribution suitable for biodiesel synthesis. Importantly, as a marine diatom possessing a silica-based frustule, *S. costatum* may exhibit distinct dielectric and structural responses under microwave irradiation compared with freshwater microalgae, potentially influencing lipid accessibility and conversion dynamics.

Despite increasing interest in microwave-assisted biodiesel production, the nonlinear interaction between microwave power, reaction time, and biomass-to-solvent ratio in marine diatoms remains insufficiently quantified. We hypothesize that microwave irradiation induces synergistic dielectric and structural effects in *Skeletonema costatum*, leading to nonlinear enhancement of in-situ lipid conversion efficiency. To test this hypothesis, this study employs response surface methodology (RSM) based on a Box–Behnken Design to quantitatively evaluate interaction effects and identify statistically significant process parameters governing biodiesel yield.

MATERIALS AND METHODS

Materials

Powdered *Skeletonema costatum* was obtained from PT Spiralife Biotechnology Indonesia (Surabaya, Indonesia) and supplied as commercially dried biomass. The biomass was used as received without further pretreatment. Detailed information regarding the initial moisture content, drying method, and particle size distribution was not provided by the supplier and therefore these parameters were not determined in this study. The

lipid content of the biomass was also not experimentally determined prior to transesterification. To maintain experimental consistency, all experiments were conducted using the same biomass batch throughout the study.

Technical grade sulfuric acid (H₂SO₄, 98% purity, obtained from CV. Surya Artathama) was used as the acid catalyst. Methanol (CH₃OH, 99%, obtained from CV. Progo Mulyo) served as the primary solvent, and n-hexane (C₆H₁₄, 96%, obtained from CV. Progo Mulyo) was used as a co-solvent.

METHODS

In-situ transesterification procedure

Preparation of reaction mixture

The reaction mixture consisted of methanol and n-hexane in a 2:1 volumetric ratio, with a total solvent volume of 150 mL (100 mL methanol and 50 mL n-hexane). Dried *Skeletonema costatum* biomass was added at specified biomass-to-solvent ratios (1:30, 1:40, and 1:50 g/mL). Sulfuric acid (technical grade, 98%) was added as a catalyst at a volume ratio of 1:100 (v/v) relative to methanol. The mixture was initially homogenized using magnetic stirring for 15 min at ambient temperature.

Microwave-assisted in-situ transesterification

The prepared mixture was transferred into a triple-neck round-bottom flask equipped with a condenser and magnetic stirring system. Microwave heating was applied at controlled power levels of 100, 300, and 600 W for 2, 5, or 8 minutes depending on the experimental design. The ranges and levels of the process variables investigated in this study are summarized in Table 1. The microwave used was Samsung Microwave Oven Solo Healty MS30T5018UK operating at 2.45 GHz.

The mixture temperature during irradiation was monitored using an external thermocouple inserted directly into the flask. Due to the heating characteristics of microwave extraction, sudden temperature spikes were observed, ranging from 50–160 °C, with both the magnitude and frequency of the spikes increasing at higher power levels. After irradiation, the reaction mixture was allowed to cool naturally to room temperature.

Product separation and purification

Following reaction completion, the mixture was filtered to remove residual solid biomass. The resulting filtrate was not subjected to any further treatment/observation such as phase separation, washing, and neutralization, and was directly treated in a Labtech EV400 VAC rotary evaporator at 50 °C under reduced pressure to remove excess methanol and n-hexane. The recovered biodiesel fraction was weighed and stored in amber glass containers at room temperature to prevent photo-oxidative degradation.

Biodiesel yield determination

Biodiesel yield was calculated according to:

$$\text{Yield (\%)} = \frac{\text{mass of product} \times \text{FAME fraction}}{\text{initial biomass mass}} \times 100 \quad (1)$$

Biodiesel yield was calculated based on the FAME content of the product, as determined by gas chromatography–mass spectrometry (GC–MS), with the initial biomass mass corresponding to the weight of *Skeletonema costatum* used at the beginning of the process, with each condition conducted in a single run.

GC–MS analysis

The chemical composition of biodiesel was analyzed using a Shimadzu GC–MS QP2010SE equipped with an Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas, with a column flow rate of 0.91 mL/min and a total flow of 21.1 mL/min. The injection was performed with a split ratio of 20:1.

The oven temperature program was set at an initial temperature of 70 °C and held for 3 min, followed by a ramp of 10 °C/min to 150 °C with a holding time of 7 min, and then further increased at 10 °C/min to 300 °C and held for 10 min.

Mass spectrometric detection was carried out using electron ionization at 70 eV. The FAME constituents were identified by matching the acquired mass spectra with those in the Wiley mass spectral library database.

Experimental design and statistical analysis

Minitab v22.3 was used to construct response surface methodology (RSM) based on a

Table 1. Variation of the parameters studied

Parameters	Variation	Remarks
Microwave power (W)	100, 300, 600	-
Irradiation time (minutes)	2, 5, 8	-
Biomass-to-solvent ratio (g/mL)	1:30, 1:40, 1:50	-
Methanol-to-n-hexane ratio	2:1	Kept constant
Catalyst volume (mL)	1	Kept constant

Box-Behnken Design (BBD) to evaluate the effects of three independent variables: microwave power (100–600 W), irradiation time (2–8 min), and biomass-to-solvent ratio (1:30–1:50 g/mL). The experimental design consisted of 15 runs, as described by Slimani et al. (2025), allowing evaluation of linear, quadratic, and interaction effects among variables. Model adequacy was evaluated using regression analysis and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Table 2 presents the experimental runs generated by the BBD along with the corresponding biodiesel yield.

Effect of microwave power

The effect of each variable studied are explained on an individual basis.

The microwave power variables used in this study were 100, 300, and 600 W. Microwave power acts as a driving force to break the structure of the algae cell membrane, so that the oil can diffuse out and dissolve in the solvent. Consequently, an increase in power generally results in enhanced yield and a reduction in extraction time (Hu et al., 2008). As illustrated in Figure 1, there is a decline in biodiesel yield output evident in the range of 100 to 300 W. This phenomenon can be attributed to the fact that as the microwave power is reduced, there is a decrease in the generation of heat. The average temperature created at 100 W power is lower than the temperature at 300 W power. The elevated temperatures within the microwave can accelerate the process of solvent evaporation, resulting in less extract being produced (Luviana et al., 2023). There's an increase in yield from 300 W to 600 W. This phenomenon can be attributed to the fact that, at elevated power levels, the microalgae surface becomes fragile and broken, increasing the dissolution of

Table 2. Designed experimental variable levels and their corresponding experimental yields

Run	Microwave power	Microalgae-to- solvent ratio (g/ml)	Time	Biodiesel yield (%)
1	100	30	5	20.99
2	600	30	5	65.58
3	100	50	5	77.30
4	600	50	5	96.17
5	100	40	2	62.95
6	600	40	2	57.67
7	100	40	8	70.23
8	600	40	8	71.56
9	300	30	2	35.17
10	300	50	2	43.36
11	300	30	8	25.76
12	300	50	8	32.59
13	300	40	5	36.46
14	300	40	5	23.16
15	300	40	5	9.37

Note: The influence of each studied variable on the response will be studied individually.

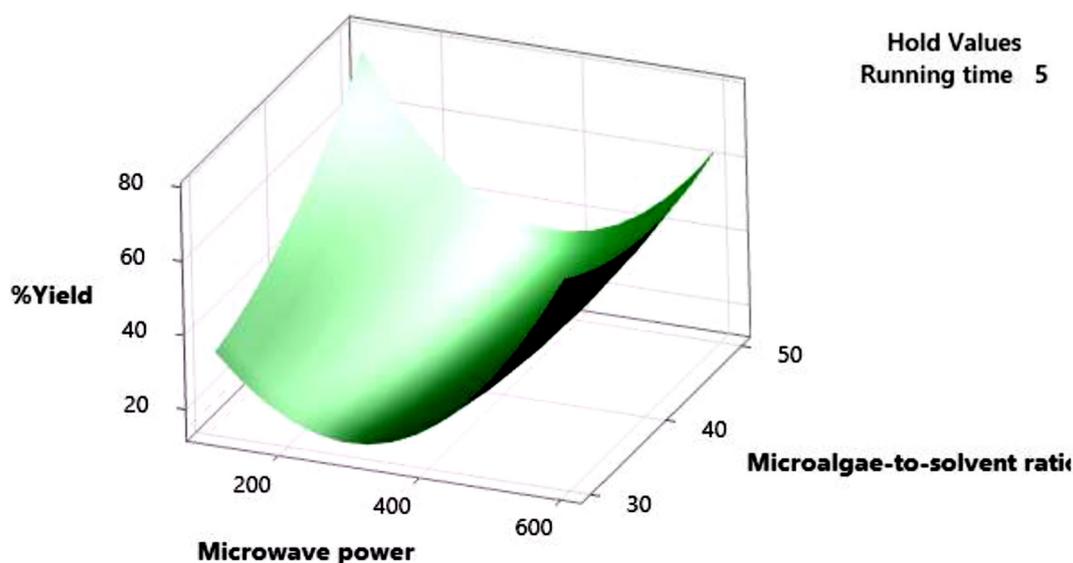


Figure 1. Effect of microwave power on the yield of *Skeletonema costatum*

the microalgae in the solvent. This, in turn, has been shown to increase the diffusion rate of the components, thereby accelerating the extraction rate and increasing the biodiesel yield (Wani and Patidar, 2025).

Effect of running time

The time variables used in this study were 2, 5, and 8 minutes. Time refers to the duration of the lipid formation reaction as well as the transesterification reaction into biodiesel due to the in-situ nature of the method used.

As illustrated in Figure 2, there is a consistent upward trend from 2 minutes to 8 minutes. This phenomenon can be attributed to the fact that as time progresses, there is an increase in the

conversion of the substrate to triglycerides (lipids), which, in turn, results in a direct increase in the biodiesel yield output. Furthermore, an increase in extraction time leads to an enhancement in the efficiency of the extraction process, attributable to an increase in the duration of solvent-material contact (Mora et al., 2024).

Effect of microalgae-to-solvent ratio

The microalgae-to-solvent ratio variable (g/mL) used in this study were 1:30, 1:40, 1:50 g of dry microalgae / ml of total solvent (n-hexane + methanol).

As demonstrated in Figure 3, an upward trend is evident as the ratio employed increases. The enhancement in biodiesel yield output is

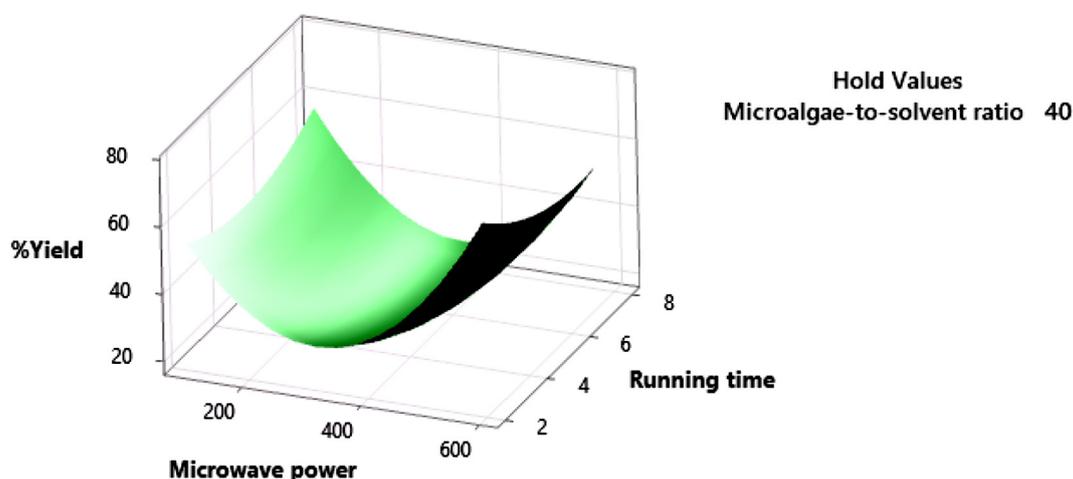


Figure 2. Effect of running time on yield of *Skeletonema costatum*

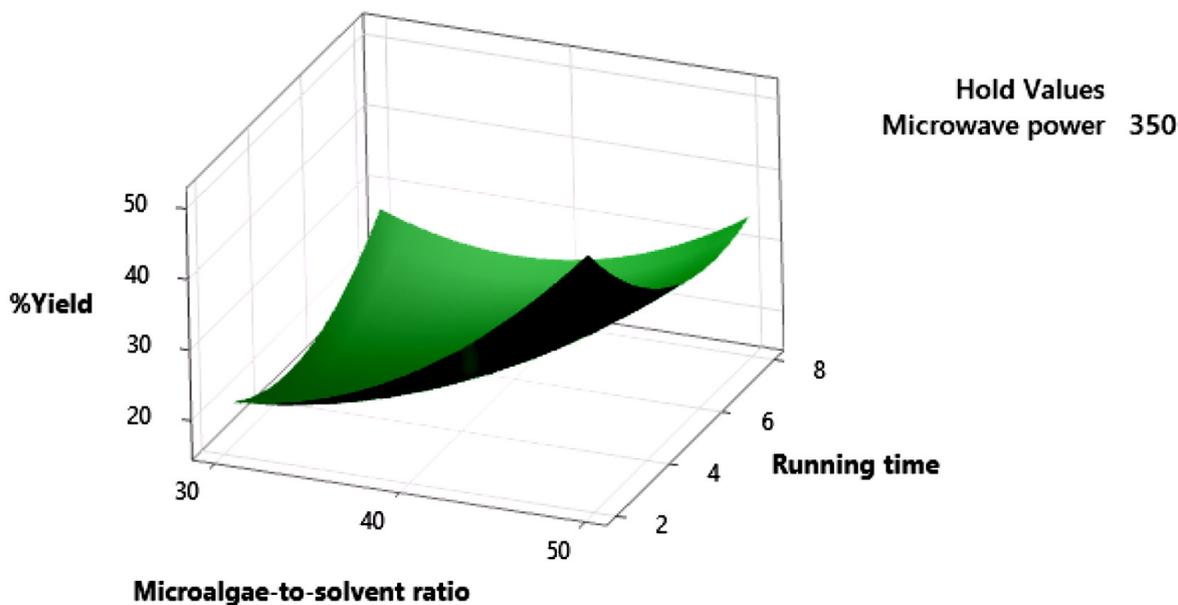


Figure 3. Effect of microalgae-to-solvent ratio on yield of *Skeletonema costatum*

attributable to the rise in the amount of methanol utilized in the transesterification reaction. This phenomenon, underpinned by the principle of stoichiometry, signifies that the addition of more methanol will shift the reaction equilibrium to the right, thereby promoting greater methyl ester formation. Consequently, the presence of excess methanol functions as a catalyst, accelerating the reaction rate and enhancing the conversion of triglycerides into biodiesel. This, in turn, leads

to an increase in the overall yield of biodiesel. Furthermore, an elevated proportion of dry microalgae in relation to the solvent suggests an increase in the surface area of contact between the two phases. This increased surface contact is a critical factor in facilitating the diffusion process, where the solvent can penetrate more effectively into the cell matrix of the dried microalgae. This enhanced penetration enables the extraction of target components, such as lipids,

Table 3. Analysis of variance (ANOVA) of experimental results

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value
Model	9	5564.56	618.28	0.97	0.544
Linear	3	1044.34	348.11	0.55	0.671
Microwave power	1	288.43	288.43	0.45	0.531
Microalgae-to-solvent ratio	1	586.65	586.65	0.92	0.381
Running time	1	59.46	59.46	0.09	0.772
Square	3	3050.66	1016.89	1.60	0.301
Microwave power	1	2950.21	2950.21	4.64	0.084
Microalgae-to-solvent ratio	1	152.79	152.79	0.24	0.645
Running time	1	182.36	182.36	0.29	0.615
2-way-interaction	3	1058.86	352.95	0.55	0.667
Microwave power*microalge-to-solvent ratio	1	569.46	569.46	0.90	0.387
Microwave power*running time	1	81.33	81.33	0.13	0.735
Microalgae-to-solvent ratio*running time	1	91.90	91.90	0.14	0.719
Error	5	3180.14	636.03		
Lack-of-fit	4	3085.12	771.28	8.12	0.257
Pure error	1	95.02	95.02		
Total	14	8744.70			

to occur in a more efficient manner (Mohammadzadeh Shirazi et al., 2017).

Analysis of variance (ANOVA) and model equation

In this research, the influence of microwave power, running time, and the ratio of microalgae-to-solvent (g/ml) on the biodiesel yield derived from *Skeletonema costatum* was investigated. The study encompassed 15 experimental runs, each conducted under varying conditions. ANOVA is used to assess the importance of each component in the model, including the independent variables' main effects and their interactions with the dependent variables (Chimklin et al., 2025). The following equation is derived from the individual parameters and their impact:

$$\begin{aligned} \text{Yield (\%)} = & 63 - 0.146 \times \text{Microwave power} - \\ & - 1.9 \times \text{Microalgae-to-solvent ratio} + 0.3 \times \\ & \times \text{Running time} + 0.000546 \times \text{Power}^2 + 0.067 \times \\ & \times \text{Microalgae-to-solvent ratio}^2 + 0.81 \times \\ & \times \text{Running time}^2 - 0.00463 \times \text{Microwave power} \times \\ & \times \text{Microalgae-to-solvent ratio} - 0.0058 \times \\ & \times \text{Microwave power} \times \text{Running time} - \\ & - 0.182 \times \text{Microalgae-to-solvent ratio} \times \\ & \times \text{Running time} \end{aligned} \quad (2)$$

Table 3 shows the results of the ANOVA to indicate the significance level of each variable and its effect on the biodiesel yield output. The ANOVA approach predicts the reproducibility of the experimental results by presenting the variation in each run in terms of biodiesel yield output. This is achieved by explaining the similarities and differences between each run (Mohammed Raffic et al., 2023). In general, the statistical significance of a model is determined by the presence of two critical values: a low p-value and a high F-value (Tripathi et al., 2009). For a variable to be deemed significant in the output, its p-value must be less than 0.05 (Nasiri and Majdi, 2023). In this experiment, the only significant variable was Microwave power². This is shown by the f-value of 4.64 which means that it is a mildly significant variable on the output (Chen et al., 2021). Whereas low f-value of other variables means its either insignificant or have less impact on the output (Tefera et al., 2025).

CONCLUSIONS

The results of study confirm that biodiesel yield is governed by significant quadratic and interaction effects, with the squared microwave

power term identified as the dominant contributor to model variance, demonstrating nonlinear enhancement of conversion efficiency.

Under optimized conditions (600 W, 5 min, 1:50 g/mL), a maximum biodiesel yield of 96.169% (dry biomass basis) was obtained. Statistical modelling using Box–Behnken design established a second-order polynomial relationship describing the process behaviour and enabled identification of statistically significant operational domains. These findings validate the hypothesis that microwave irradiation induces synergistic process intensification beyond linear parameter scaling.

GC–MS analysis determined a FAME content of 79.53%, dominated by hexadecanoic acid methyl ester, confirming successful transesterification and establishing the fatty acid profile of microwave-derived biodiesel from this marine diatom species.

This work provides the first systematic quantitative model describing microwave-assisted in-situ biodiesel conversion in *Skeletonema costatum* and demonstrates that nonlinear dielectric–process interactions can be exploited to significantly reduce reaction time while maintaining high ester yield. The developed regression framework enables predictive optimization of operational parameters in marine microalgal systems.

Acknowledgements

The authors wish to thank the Department of Chemical Engineering, Faculty of Industrial Engineering, Universitas Pembangunan Nasional Veteran Yogyakarta for supporting and funding this work.

REFERENCES

1. Al-Humairi, S. T., Lee, J. G. M., Salihu, M., Harvey, A. P. (2022). Biodiesel Production through acid catalyst in situ reactive extraction of *Chlorella vulgaris* foamate. *Energies*, 15(12), 4482. <https://doi.org/10.3390/en15124482>
2. Baqi, F., Putri, R. S. I., Mirzayanti, Y. W. (2022). Proses pembuatan biodiesel dari mikroalga *Nannochloropsis* sp. menggunakan metode transesterifikasi in-situ dengan katalis KOH. *Equilibrium Journal of Chemical Engineering*, 6(2), 92. <https://doi.org/10.20961/equilibrium.v6i2.63257>
3. Barnard, T. M., Leadbeater, N. E., Boucher, M. B., Stencel, L. M., Wilhite, B. A. (2007).

- Continuous-flow preparation of biodiesel using microwave heating. *Energy and Fuels*, 21(3), 1777–1781. <https://doi.org/10.1021/ef0606207>
4. Chen, W. H., Chiu, G. L., Chyuan Ong, H., Shiung Lam, S., Lim, S., Sik Ok, Y., E. Kwon, E. (2021). Optimization and analysis of syngas production from methane and CO₂ via Taguchi approach, response surface methodology (RSM) and analysis of variance (ANOVA). *Fuel*, 296(March), 120642. <https://doi.org/10.1016/j.fuel.2021.120642>
 5. Chimklin, K., Phuangkaew, S., Deeying, J. (2025). Multi-objective optimization of laser spot welding parameters for enhancing mechanical properties of hard disk components using response surface methodology. *Results in Engineering*, 25(December 2024), 104449. <https://doi.org/10.1016/j.rineng.2025.104449>
 6. Dianursanti, Religia, P., Wijanarko, A. (2015). Utilization of n-Hexane as co-solvent to increase biodiesel yield on direct transesterification reaction from marine microalgae. *Procedia Environmental Sciences*, 23(Ictcred 2014), 412–420. <https://doi.org/10.1016/j.proenv.2015.01.059>
 7. Ehimen, E. A., Sun, Z. F., Carrington, C. G. (2010). Variables affecting the in situ transesterification of microalgae lipids. *Fuel*, 89(3), 677–684. <https://doi.org/10.1016/j.fuel.2009.10.011>
 8. Galusnyak, S. C., Petrescu, L., Cosprundan, A. M., Cormos, C. C. (2024). Biodiesel production using various methanol sources and catalytic routes: A techno-environmental analysis. *Renewable Energy*, 232(June), 121051. <https://doi.org/10.1016/j.renene.2024.121051>
 9. Go, A. W., Sutanto, S., Ong, L. K., Tran-Nguyen, P. L., Ismadji, S., Ju, Y. H. (2016). Developments in in-situ (trans) esterification for biodiesel production: A critical review. *Renewable and Sustainable Energy Reviews*, 60, 284–305. <https://doi.org/10.1016/j.rser.2016.01.070>
 10. Hu, Z., Cai, M., Liang, H. H. (2008). Desirability function approach for the optimization of microwave-assisted extraction of saikosaponins from *Radix Bupleuri*. *Separation and Purification Technology*, 61(3), 266–275. <https://doi.org/10.1016/j.seppur.2007.10.016>
 11. Huschek, G., Rawel, H. M., Schweikert, T., Henkel-Oberländer, J., Sagu, S. T. (2022). Characterization and optimization of microwave-assisted extraction of B-phycoerythrin from *Porphyridium purpureum* using response surface methodology and Doehlert design. *Bioresource Technology Reports*, 19(September). <https://doi.org/10.1016/j.biteb.2022.101212>
 12. Kalsum, U., Kusuma, H. S., Roesyadi, A., Mahfud, M. (2018). Production biodiesel via in-situ transesterification from *Chlorella* sp. Using Microwave with base catalyst. *Korean Chemical Engineering Research*, 56(5), 773–778. <https://doi.org/10.9713/kcer.2018.56.5.773>
 13. Katili, V. R. A., Kawaroe, M., Prartono, T. (2012). *Komposisi Asam Lemak Mikroalga Jenis Skeletomena costatum, Thalassiosira sp., dan Chaetoceros gracilis*. [Institut Pertanian Bogor]. <http://repository.ipb.ac.id/handle/123456789/57985>
 14. Li, X., Xu, H., Wu, Q. (2007). Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnology and Bioengineering*, 98(4), 764–771. <https://doi.org/10.1002/bit.21489>
 15. Luviana, A., Putri, A., Alatif, I. A., Nurulgina, R., Permatasari, R. P., Sihombing, R. P., Paramitha, T. (2023). Pengaruh pelarut dan daya microwave terhadap hasil ekstrak daun pepaya dengan metode microwave assisted extraction. *Prosiding Industrial Research Workshop and National Seminar*, 14(1), 213–217. <https://doi.org/10.35313/irwns.v14i1.5388>
 16. Ma, B., Wang, A. (2025). Exploring the role of renewable energy in green job creation and sustainable economic development: An empirical approach. *Energy Strategy Reviews*, 58(June 2024), 101642. <https://doi.org/10.1016/j.esr.2025.101642>
 17. Mardhiah, H. H., Ong, H. C., Masjuki, H. H., Lim, S., Lee, H. V. (2017). A review on latest developments and future prospects of heterogeneous catalyst in biodiesel production from non-edible oils. *Renewable and Sustainable Energy Reviews*, 67, 1225–1236. <https://doi.org/10.1016/j.rser.2016.09.036>
 18. Mohamadzadeh Shirazi, H., Karimi-Sabet, J., Ghotbi, C. (2017). Biodiesel production from *Spirulina* microalgae feedstock using direct transesterification near supercritical methanol condition. *Bioresource Technology*, 239, 378–386. <https://doi.org/10.1016/j.biortech.2017.04.073>
 19. Mohammed Raffic, N., Mohammed Khondoker, A., Mohammed Ali Kaabi, A., Ali Hamad Majrashi, A., Yhaya Mohammed Qusadi, I., Ibrahim Mohamed Moawad, F., Hadidi, H., Tharwan, M., & Saminathan, R. (2023). Utilization of ANOVA analysis in identifying the effects of various parameters on the corrosion behaviour of 7021 Al alloys in simulated RED SEA conditions. *Materials Today: Proceedings*, xxx. <https://doi.org/10.1016/j.matpr.2023.06.278>
 20. Mora, J. M. R., Lacson, C. F. Z., Choi, A. E. S., Chung, T. W., Retumban, J. D., Abarca, R. R. M., Grisdanurak, N., de Luna, M. D. G. (2024). Biodiesel production from soybean oil via LiOH-pumice catalytic transesterification and BBD-RSM optimization. *Energy Reports*, 11(April), 4032–4043. <https://doi.org/10.1016/j.egyr.2024.03.050>
 21. Narayanan, M. (2024). Marine algae biomass: A viable and renewable resource for biofuel production:

- A review. *Algal Research*, 82(January), 103687. <https://doi.org/10.1016/j.algal.2024.103687>
22. Naseef, H. H., Tulaimat, R. H. (2025). Transesterification and esterification for biodiesel production: A comprehensive review of catalysts and palm oil feedstocks. In *Energy Conversion and Management: X26*, October 2024. Elsevier Ltd. <https://doi.org/10.1016/j.ecmx.2025.100931>
 23. Nasiri, M., Majdi, H. (2023). Adsorption refrigeration optimization via response surface methodology using waste heat in a ship. *Iranian Journal of Science and Technology - Transactions of Mechanical Engineering*, 47(4), 1449–1466. <https://doi.org/10.1007/s40997-023-00598-1>
 24. Nirmala, N., Dawn, S. S. (2021). Optimization of *Chlorella variabilis*. MK039712.1 lipid transesterification using Response Surface Methodology and analytical characterization of biodiesel. *Renewable Energy*, 179, 1663–1673. <https://doi.org/10.1016/j.renene.2021.07.123>
 25. Pekkoh, J., Ruangrit, K., Aurepatipan, N., Duangjana, K., Sensupa, S., Pumas, C., Chaichana, C., Pathom-aree, W., Kato, Y., Srinuanpan, S. (2024). CO₂ to green fuel converter: Photoautotrophic-cultivation of microalgae and its lipids conversion to biodiesel. *Renewable Energy*, 222(July 2023), 119919. <https://doi.org/10.1016/j.renene.2023.119919>
 26. Pérez, L., Salgueiro, J. L., González, J., Parralejo, A. I., Maceiras, R., Cancela, Á. (2017). Scaled up from indoor to outdoor cultures of *Chaetoceros gracilis* and *Skeletonema costatum* microalgae for biomass and oil production. *Biochemical Engineering Journal*, 127, 180–187. <https://doi.org/10.1016/j.bej.2017.08.016>
 27. Rengga, W. D. P., Prayoga, A. B., Asnafi, A., Triwibowo, B. (2019). Ekstraksi Minyak mikro-algae skeletonema costatum dengan bantuan gelombang ultrasonik. *Jurnal Rekayasa Bahan Alam Dan Energi Berkelanjutan*, 3(1), 1–5.
 28. Sanjurjo, C., Oulego, P., Bartolomé, M., Rodríguez, E., Gonzalez, R., Hernández Battez, A. (2024). Biodiesel production from the microalgae *Nannochloropsis gaditana*: Optimization of the transesterification reaction and physicochemical characterization. *Biomass and Bioenergy*, 185(April). <https://doi.org/10.1016/j.biombioe.2024.107240>
 29. Scott, S. A., Davey, M. P., Dennis, J. S., Horst, I., Howe, C. J., Lea-Smith, D. J., Smith, A. G. (2010). Biodiesel from algae: Challenges and prospects. *Current Opinion in Biotechnology*, 21(3), 277–286. <https://doi.org/10.1016/j.copbio.2010.03.005>
 30. Slimani, C., Fadil, M., Rais, C., Ullah, R., Iqbal, Z., Santanatoglia, A., Caprioli, G., Benjelloun, M., Lazraq, A., Bouyahya, A. (2025). Optimization of phenolic compounds extraction and antioxidant activity from Moroccan *Crocus sativus* L. by-products using predictive modeling and Box-Behnken design. *Microchemical Journal*, 212(October 2024), 113444. <https://doi.org/10.1016/j.microc.2025.113444>
 31. Tefera, N. T., Nallamothu, R. B., Lakew, G. A., Kurse, T. K. (2025). Optimization of biodiesel production from cottonseed oil using response surface methodology and artificial neural network techniques. *Scientific African*, 28(January), e02665. <https://doi.org/10.1016/j.sciaf.2025.e02665>
 32. Tripathi, P., Srivastava, V. C., Kumar, A. (2009). Optimization of an azo dye batch adsorption parameters using Box-Behnken design. *Desalination*, 249(3), 1273–1279. <https://doi.org/10.1016/j.desal.2009.03.010>
 33. Umamaheswari, J., Kavitha, M. S., Shanthakumar, S. (2020). Outdoor cultivation of *Chlorella pyrenoidosa* in paddy-soaked wastewater and a feasibility study on biodiesel production from wet algal biomass through in-situ transesterification. *Biomass and Bioenergy*, 143, 105853. <https://doi.org/10.1016/j.biombioe.2020.105853>
 34. Wani, K. M., Patidar, R. (2025). Microwave-assisted extraction of pectin from lemon peel powder: Optimization and physicochemical properties. *Sustainable Chemistry for the Environment*, 9(February), 100223. <https://doi.org/10.1016/j.sscenv.2025.100223>
 35. Widyastuti, C. R., Dewi, A. C. (2015). Sintesis biodiesel dari minyak mikroalga *Chlorella Vulgaris* dengan reaksi transesterifikasi menggunakan katalis koh. *Jurnal Bahan Alam Terbarukan*, 4(1), 29–33. <https://doi.org/https://doi.org/10.15294/jbat.v3i1.3099>
 36. Zahedi, A., Moradi, Z., Molaeimanesh, G., MansoorGhanaei, M. J. (2024). Experimental development of biodiesel fuel derived from freshwater microalgae for improved engine performance and reduced emissions. *Energy Reports*, 12(September 2023), 6036–6045. <https://doi.org/10.1016/j.egy.2024.11.084>