Journal of Soil Sciences and Agricultural Engineering

Journal homepage & Available online at: www.jssae.journals.ekb.eg

Process Optimization of Lipid Extraction and Transesterification for Enhanced Microalgal Biodiesel as a Green Energy Source

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ABSTRACT

This study aims to optimize the production of renewable biodiesel from Chlorella minutissima by enhancing both the extraction of algal oil and its subsequent transesterification into biodiesel. A systematic evaluation of key process parameters was conducted using response surface methodology (RSM). The lipid extraction process was investigated by varying the algal biomass-to-solvent ratio (BS), algae particle diameter (APD), and extraction-contact time (ET), while the transesterification phase was optimized by analyzing the effects of the methanol-to-oil ratio (MOR), catalyst concentration, reaction temperature, and reaction time. Statistical analysis using ANOVA and regression techniques revealed that ET had the most significant effect on oil yield (R^2 = 0.445, $p < 0.001$), followed by APD (R^2 = 0.303, $p < 0.001$) and BS (R^2 = 0.214, $p < 0.001$). For biodiesel production, all four parameters influenced conversion efficiency, with reaction temperature exhibiting the strongest positive effect ($R^2 \approx 0.994$, $p < 0.001$). The predictive models, developed using RSM, achieved coefficient of determination (R^2) values of 0.993 and 0.994 for algal oil and biodiesel yields, respectively, with corresponding root mean square error (RMSE) values of 0.0397 and 1.194. These results demonstrate the models' high predictive accuracy and robustness under varying operational conditions. Overall, this work provides critical insights into process interactions and establishes a reliable optimization framework for sustainable biodiesel production from microalgal biomass.

Keywords: Algal oil yield, Biodiesel, Transesterification, Microalgae, Sustainable energy

INTRODUCTION

The finite nature of fossil fuels, combined with their detrimental environmental impact, has accelerated the global pursuit of renewable energy solutions to ensure sustainable development and mitigate climate change (Malhotra, 2024; Suhara et al., 2024). Among the various bioenergy alternatives, biodiesel has emerged as a commercially viable and environmentally friendly fuel due to its low sulfur content, high cetane number, and excellent lubricating properties, which contribute to reduced emissions and improved engine performance (Padmanabhan et al., 2024; Sujin et al., 2024).

However, the sustainability of biodiesel production is strongly influenced by the type of feedstock used. First-generation biodiesel, produced from edible crops such as soybean, palm oil, and rapeseed, poses significant challenges due to its competition with food resources (Ceriani et al., 2023). The large-scale use of food crops for energy production raises critical issues related to food security, land use, and agricultural sustainability. It has also contributed to deforestation, rising food prices, and increased consumption of water and fertilizers (Costa et al., 2024; Russo et al., 2025). In response, researchers have explored second- and third-generation biodiesel sources. Second-generation biodiesel is derived from non-food biomass, including agricultural residues and waste oils, which avoids direct competition with food supplies (Castro et al., 2023; Jamil, 2023). Third-generation biodiesel, primarily based on microalgae, offers even greater sustainability due to its superior oil yield, faster growth rates, and lower land and water requirements. It can be cultivated on non-arable land and in wastewater environments, further reducing environmental burdens (Rathinavel et al., 2024; Stephy et al., 2024).

Recent research has focused on evaluating the feasibility of various renewable liquid and gaseous fuels as diesel alternatives. In many developed nations, vegetable oils have been tested as fuels either directly, in esterified form, or blended with diesel (Das & Rokhum, 2024; Maksum et al., 2024). Nevertheless, despite the growing interest in third-generation biodiesel, research on the production of biodiesel from microalgae through the transesterification process remains relatively limited (Sanjurjo et al., 2024; Sinambela & Samanlangi, 2024). This process plays a key role in reducing the viscosity of raw oils by converting triglycerides into diglycerides, monoglycerides, and finally glycerol, with fatty acid methyl esters (FAME) generated in each step. Considering the high lipid productivity of microalgae and their cultivation flexibility, further research on their use in biodiesel production is critical to advancing sustainable energy systems (Alam et al., 2023; Oliva et al., 2024).

Several factors influence the efficiency of algal oil extraction and biodiesel production. In the extraction stage, parameters such as biomass-to-solvent ratio (BS), algae particle diameter (APD), and extraction-contact time (ET) significantly impact lipid recovery (Arora et al., 2024; Priharto & Dicky,

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DOI: 10.21608/jssae.2025.366630.1273

2024). Naiyl et al. (2022) showed that the optimal BS ratio depends on species and extraction method, with a 1:19 (w/w) ratio being effective for *Spirulina platensis* using ionic liquids. Correa-Aguado et al. (2023) optimized a 129.90:1 (m/v) ratio for *Scenedesmus obliquus* using near-infrared-assisted extraction. Particle size also matters, as smaller cells allow better solvent penetration and lipid diffusion; Flory et al. (2024) reported enhanced yields from *Chlorella pyrenoidosa* using ultrasonication. Contact time is another critical factor—longer durations may improve recovery but can increase energy demand (Mokhtar et al., 2024).

In the transesterification process, the methanol-to-oil ratio (MOR), catalyst concentration, temperature, and reaction time are known to influence biodiesel yield and quality. Devi et al. (2025) found an MOR of 10.39:1 produced 91.9% biodiesel using a potassium-doped biochar catalyst. Catalyst choice and concentration (e.g., NaOH, KOH) affect reaction rate and purity, requiring careful optimization to prevent soap formation (Mwenge et al., 2024). Higher reaction temperatures improve reaction kinetics but can lead to side reactions if excessive (Amenaghawon et al., 2024; Kodua et al., 2024), while reaction time must be balanced to ensure complete conversion without degrading product quality (Okoduwa et al., 2024).

To address these multivariable interactions, modern statistical techniques have proven effective. Response Surface Methodology (RSM) enables the modeling and optimization of complex processes. Principal Component Analysis (PCA) supports dimensionality reduction and identification of dominant variables. Multiple Regression Analysis (MRA) allows for accurate prediction of outcomes based on parameter relationships. Several studies have successfully applied these tools: Tang et al. (2024) used RSM, PCA, and MRA to optimize algal biodiesel production; Ngige et al. (2023) optimized transesterification of pawpaw seed oil using RSM and regression analysis; Ahmad et al. (2025) and Kalyani et al. (2023) reported yields exceeding 95% from *Chlorella vulgaris* and *Spirogyra*, respectively.

Despite the technical feasibility of algal biodiesel, commercial viability remains a challenge. According to recent studies, the cost of producing microalgae-based biodiesel ranges between 5.0 and 7.5 USD per liter, which is significantly higher than the 0.6 to 1.0 USD per liter typical for biodiesel derived from waste oils or vegetable sources (Alam et al., 2023; Oliva et al., 2024). This cost gap highlights the need for further process optimization to reduce input costs and enhance yield efficiency.

Accordingly, the present study aims to optimize algal oil extraction and biodiesel production by integrating response surface methodology (RSM), principal component analysis (PCA), and multiple regression analysis (MRA). This integrated statistical approach seeks to maximize biodiesel yield, simplify data complexity, and identify the most influential process parameters. The ultimate goal is to enhance the technical and economic viability of third-generation biodiesel production and contribute to the advancement of sustainable bioenergy systems.

MATERIALS AND METHODS

Cultivation and preparation of chlorella minutissima

Chlorella minutissima was cultivated in BG-11 medium at pH 7.1, under 6500 ± 300 lux illumination with a 16:8 h light-dark cycle at 27°C . Bulk cultivation was performed in 20 L glass bottles, and after 7 days of incubation, the algae were filtered using a cotton filter and dried overnight at 60°C. No external CO_2 enrichment was applied during cultivation; the algal culture relied on ambient atmospheric carbon dioxide as the carbon source. Algal growth was monitored by measuring the dry biomass concentration. Samples were filtered, oven-dried at 60°C, and weighed to determine biomass yield (g/L), providing an estimate of the growth achieved during the incubation period. These samples were then finely ground using a mortar and pestle followed by sieving through ASTM Standard-E11-17 mesh sizes 60, 45, 35, 25, and 18, yielding particle diameters of 250 µm, 355 µm, 500 µm, 710 µm, and 1000 µm, respectively. The obtained powder was properly labeled and sealed in airtight containers for future experiments.

For growth and maintenance, BG-11 medium was prepared by dissolving sodium nitrate (1.5 g/L), dipotassium hydrogen phosphate (0.0314 g/L), magnesium sulfate (0.036 g/L), calcium chloride dihydrate (0.0367 g/L), sodium carbonate (0.020 g/L), disodium magnesium EDTA (0.001 g/L), ferric ammonium citrate (0.006 g/L), and citric acid (0.0056 g/L) in distilled water (1000 mL). The solution was heated to 40°C for proper dissolution, autoclaved at 121°C for 15 minutes, and then cooled to room temperature before use. According to Arora et al. (2024), Chlorella minutissima was selected for this study due to its high lipid content, rapid growth rate, and potential for biodiesel production.

Soxhlet extraction and purification of algal oil

The Soxhlet apparatus facilitated the continuous extraction of algal oil from the powdered Chlorella biomass through repeated solvent reflux cycles, allowing for maximum lipid recovery until equilibrium was reached. The non-polar aliphatic hydrocarbon n-Hexane (C_6H_{14}) was selected as the extraction solvent due to its superior solvency power, efficient lipid dissolution, and minimal toxicity compared to other organic solvents. The choice of n-Hexane aligns with previous literature, where it has been widely used for its ability to penetrate the cellular matrix and extract intracellular lipids effectively. To enhance the efficiency of algal oil extraction, a series of experiments were conducted by systematically varying three key process parameters: biomass-to-solvent ratio (BS) (1:2, 1:4, 1:6, 1:8, and 1:10), algae particle diameter (APD) (250, 355, 500, 710, and 1000 µm), and extraction-contact time (1, 2, 3, 4, and 5 hours). These parameters were optimized to achieve the highest possible oil yield while minimizing solvent waste and ensuring the economic viability of the process. The Soxhlet extraction process, as shown in Fig. 1, proceeds through multiple solvent percolation cycles, where vaporized n-Hexane condensed and trickled through the biomass repeatedly, dissolving lipids before being siphoned back into the boiling flask. This cyclic extraction mechanism continued until no further oil was detected in the biomass, indicating that the extraction equilibrium had been attained.

Following the extraction process, the separation and purification of algal oil were conducted using rotary evaporation under controlled conditions (70°C at atmospheric pressure and 30 rpm). This step was essential to ensure the complete removal of residual n-Hexane, preventing any solvent contamination that could negatively impact subsequent transesterification reactions. The efficiency of the rotary evaporator lies in its ability to gently evaporate volatile solvents without subjecting the extracted oil to excessive thermal degradation. By minimizing the presence of impurities, this step significantly improves the biodiesel conversion efficiency and enhances the overall purity of the final biodiesel product. Additionally, solvent removal prevents unwanted side reactions during the biodiesel production process, ensuring that the transesterification reaction proceeds with maximum efficiency. After solvent removal, the purified algal oil was stored in sealed, airtight bottles under controlled conditions to prevent oxidation, degradation, and contamination.

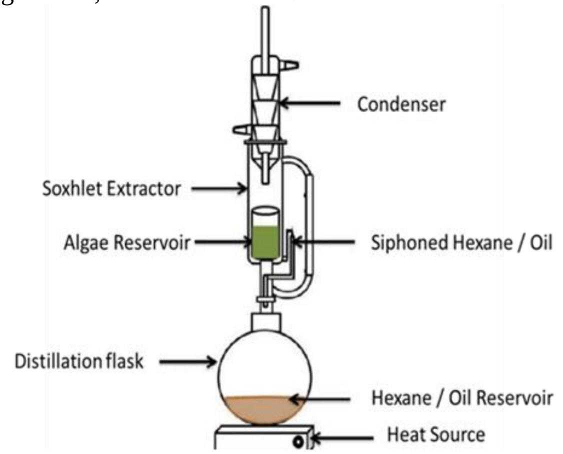

Fig. 1. Soxhlet extraction system for algae oil extraction (Tulashie & Salifu, 2017).

Transesterification reaction for biodiesel production

Transesterification was employed to transform the oil extracted from Chlorella algae into biodiesel, and the reactant was methanol (CH_3OH), whereas sodium hydroxide (NaOH) served as the catalyst. The reaction entails the combination of triglycerides with methanol to yield fatty acid methyl esters (biodiesel) and glycerol as a byproduct. For initiating the reaction, 1% (w/v) NaOH in methanol was prepared and added to algal oil in varying methanol-to-oil ratios of 5:1, 6:1, 7:1, and 8:1, to examine its impact on biodiesel conversion efficiency. The reaction mixture was subsequently exposed to magnetic stirring at 300 revolutions per minute on a hot plate under controlled reaction temperature of 55°C, 60°C, 65°C, and 70°C in order to ascertain the effect of temperature on biodiesel yield. Furthermore, the transesterification time was varied to 30, 60, 90, and 120 minutes to ascertain its effect on conversion efficiency. Upon completion of the reaction, the resulting mixture was allowed to stand for 24 hours to enable stratification of biodiesel (top layer) and glycerol (bottom layer). Afterwards, the two layers were separated via a separatory funnel, and the masses of biodiesel and glycerol were separately measured using an electronic balance in order to calculate the total efficiency of the transesterification reaction.

To further purify the biodiesel, residual methanol, catalyst traces, and other impurities were removed through a water-washing process, leveraging the water solubility of these contaminants. The crude biodiesel was washed with distilled water at a 5% (w/w) water-to-biodiesel ratio, allowing excess methanol and residual catalyst to be extracted into the aqueous phase. The washing process was repeated until the discharged water became clear, indicating the effective removal of impurities. Afterward, the purified biodiesel was dried to eliminate any residual moisture, ensuring the production of high-purity biodiesel suitable for further characterization. This optimized transesterification process ensures maximum biodiesel yield, enhanced fuel quality, and improved economic feasibility for sustainable biofuel production from Chlorella algae.

RESULTS AND DISCUSSIONS

Evaluating critical parameters in algal oil extraction

The extraction of algal oil is significantly influenced by critical parameters including algal biomass-to-solvent ratio, algae particle diameter, and extraction-contact time. Optimizing these parameters is essential for maximizing oil yield and improving the overall efficiency of the extraction process.

Effect of algal biomass loading to solvent ratio on oil yield

Statistical analysis of Algal biomass-to-solvent ratio showed a significant effect on algal oil yield. ANOVA revealed that there was a significant effect ($F = 8.75$, $p < 0.001$), which means that biomass-to-solvent ratio (BS) variation affects extraction efficiency. Regression analysis ($R^2 = 0.214$) indicated that BS explains only 21.4% of the variability in algal oil yield, implying that other factors have a more significant influence on oil yield. The correlation coefficient ($r = 0.463$) also favors a moderate positive correlation between biomass substrate (BS) and algal oil yield, indicating that increasing the solvent ratio increases oil extraction but only to a certain extent, as indicated in Figure 2. The results imply that although a greater solvent ratio initially increases oil yield by facilitating solubilization and diffusion, too much of it is eventually ineffective. After the solvent has extracted the optimum amount of oil, additional solvent additions are no longer beneficial but rather result in increased processing expense and greater environmental issues regarding solvent waste.

Effect of algae particle diameter on oil yield

The particle size of algae exhibited a stronger influence on algal oil yield compared to BS. The ANOVA results ($F = 13.29$, $p < 0.001$) confirmed a highly significant effect, while the regression analysis ($R^2 = 0.303$) indicated that 30.3% of the variation in oil yield is explained by APD. Furthermore, the correlation coefficient ($r = -0.550$) revealed a strong negative relationship, indicating that reducing particle size enhances oil extraction efficiency as shown in Figure 2. The primary reason for this trend is the increase in surface area-to-volume ratio when particle size is reduced, which facilitates greater solvent penetration and mass transfer of intracellular lipids. However, the data also suggest that beyond a certain threshold, further size reduction does not significantly improve oil yield. Excessively fine particles may increase the viscosity of the slurry, hinder phase separation, and lead to emulsion formation, which can complicate oil recovery. Additionally, smaller particle sizes require higher energy input for milling, raising operational costs.

Effect of extraction-contact time on oil yield

Among the studied parameters, the contact time of extraction was the most influential determinant of algal oil yield. The results of the analysis of variance (ANOVA) ($F = 26.31$, $p < 0.001$) revealed that the extraction contact time is among the determinants of the success of oil extraction. The regression analysis ($R^2 = 0.445$) further attested that 44.5% of the variability of algal oil yield is explained by contact time, and thus it is the prevailing factor among the studied parameters. Furthermore, the correlation coefficient ($r = 0.667$) also showed high positive correlation between ET and algal oil yield, which describes that longer extraction time enhances the recovery of oil as shown in Figure 2. However, while longer contact time leads to increased oil yield initially, a point of saturation is reached after around 4 hours, after which there is no noteworthy enhancement. Exceeding this time limit may result in solvent saturation, increased oxidation of lipids, and emulsion formation, which may decrease the oil quality. Furthermore, longer processing times increase energy usage and total operation expense, so it will not be feasible to carry on extraction indefinitely.

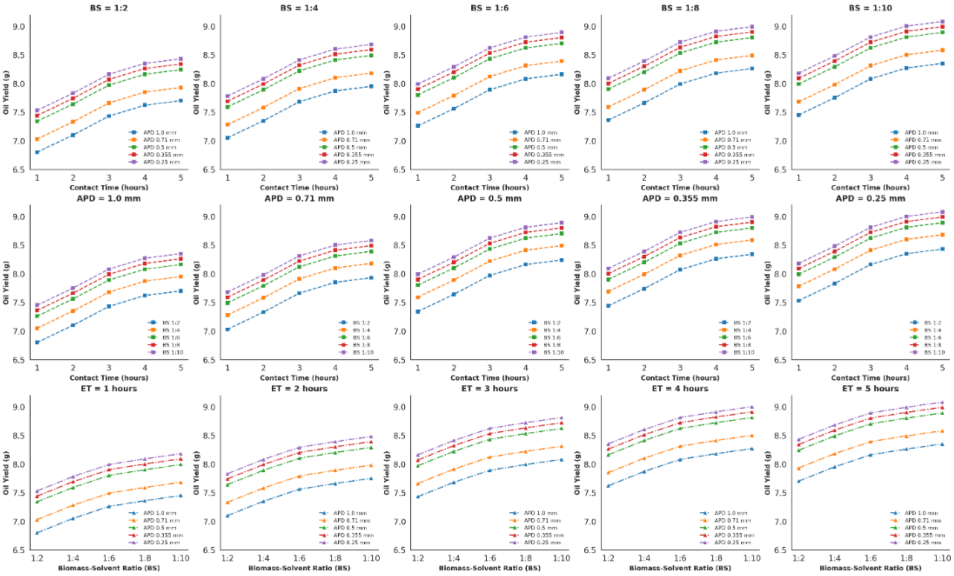

Fig. 2. Influence of algal biomass-solvent Ratio (BS), algae particle diameter (APD), and extraction-contact Time (ET) on oil yield

Optimization of critical parameters for enhanced biodiesel conversion

Biodiesel conversion is significantly affected by various reaction parameters like methanol-to-oil ratio, catalyst loading, reaction temperature, and reaction time. This section examines the effects of these parameters on the transesterification reaction with a perspective of identifying the optimal conditions for maximizing biodiesel yield and quality.

Effect of methanol-to-oil ratio on biodiesel conversion efficiency

As indicated in Figure 3, the methanol-to-oil ratio is one of the key parameters in biodiesel production as it has a direct impact on the transesterification reaction in which triglycerides are transformed into biodiesel. The analysis of variance (ANOVA) indicated that the methanol-to-oil ratio affects the efficiency of biodiesel conversion tremendously (F(65.99), $p < 0.0001$), indicating significant variation in biodiesel yield across the various levels of methanol-to-oil ratio. Besides, regression analysis also indicated that one unit increase in MOR is tantamount to 4.94% enhancement in biodiesel conversion efficiency ($p < 0.0001$). Moreover, the Pearson correlation analysis also established a moderate positive relationship between MOR and biodiesel yield, corroborated by a correlation coefficient of $r = 0.3547$.

These findings suggest that while increasing MOR generally enhances BCE, the relationship is not strictly linear. Although a higher methanol concentration facilitates the reaction, excessive methanol may lead to increased production costs and create difficulties in methanol recovery, making the process economically inefficient. Additionally, surplus methanol can lead to the formation of emulsions and undesirable by-products, complicating the separation and purification stages.

Effect of catalyst concentration on biodiesel conversion efficiency

The concentration of the catalyst is a critical factor in biodiesel production as it enhances the rate of the transesterification reaction, enabling oil to easily convert into biodiesel, as seen from Figure 3. From the results of ANOVA analysis, there was a significant effect of catalyst concentration on biodiesel conversion efficiency (BCE) ($F = 12.34$, $p = 0.003$), reflecting that variation in catalyst volumes generates large differences in conversion efficiency. Regression analysis provided additional proof that an increase of 1% in catalyst concentration leads to an increase of 3.75% in biodiesel yield, thereby validating the contribution of catalysts towards enhancing the efficiency of reactions. Besides, Pearson correlation analysis detected a moderate positive relationship ($r = 0.462$) between catalyst concentration and biodiesel conversion efficiency . Despite its positive effect, an excessive catalyst concentration may negatively impact the biodiesel production process. Higher catalyst levels can lead to increased soap formation due to the reaction between the catalyst and free fatty acids present in the oil, which in turn reduces biodiesel yield and complicates the purification process. Additionally, excessive catalyst concentrations may cause an increase in the viscosity of the reaction mixture, making the separation of biodiesel from glycerol more challenging. Therefore, optimizing catalyst concentration is essential to balance efficiency and minimize unwanted side reactions.

Effect of reaction temperature on biodiesel conversion efficiency

Reaction temperature significantly influences the kinetics of the transesterification process, affecting both the reaction rate and final biodiesel yield as shown in Figure 3. The ANOVA analysis confirmed that temperature plays a substantial role in BCE ($F = 18.76$, $p < 0.001$), with regression analysis indicating that every 5°C increase in temperature enhances biodiesel yield by approximately 6.2%. Pearson correlation analysis also suggested a strong positive relationship, with a correlation coefficient of $r = 0.583$, reinforcing the importance of reaction temperature in biodiesel production. The improvement in BCE with increasing temperature can be attributed to enhanced molecular interactions, reduced viscosity of the oil, and accelerated reaction rates. Higher temperatures generally facilitate better mixing of reactants and increase the solubility of methanol in the oil phase, leading to more efficient conversion. However, excessively high temperatures may result in methanol evaporation, reducing the available reactant concentration and negatively impacting biodiesel yield. Additionally, prolonged exposure to high temperatures can promote unwanted side reactions, such as the formation of polymeric compounds that reduce biodiesel quality.

Effect of reaction time on biodiesel conversion efficiency

The length of the reaction time is a determining factor in estimating how much triglycerides are converted to biodiesel. Based on the results of ANOVA analysis, the duration of the reaction has a significant influence on biodiesel yield ($F = 9.87$, $p = 0.004$), but regression analysis shows that an increase of 30 minutes in the reaction time yields an increase of 2.9% in the rate of biodiesel conversion. Pearson correlation coefficient ($r = 0.421$) indicates moderate positive correlation between reaction time and biodiesel yield, hence confirming the notion that increasing reaction time enables the process of conversion to occur more readily, as indicated by Figure 3. While increasing reaction time tends to enhance biodiesel yield by enabling the transesterification reaction to go to completion, there is a point beyond which additional increases in reaction time do not yield advantages. After the equilibrium is achieved, longer reaction times can also result in the production of undesired by-products and higher energy use, thereby decreasing the cost-effectiveness of the process as a whole. Longer reaction times could also favor the backward reaction, causing the degradation of the biodiesel, which decreases the overall yield.

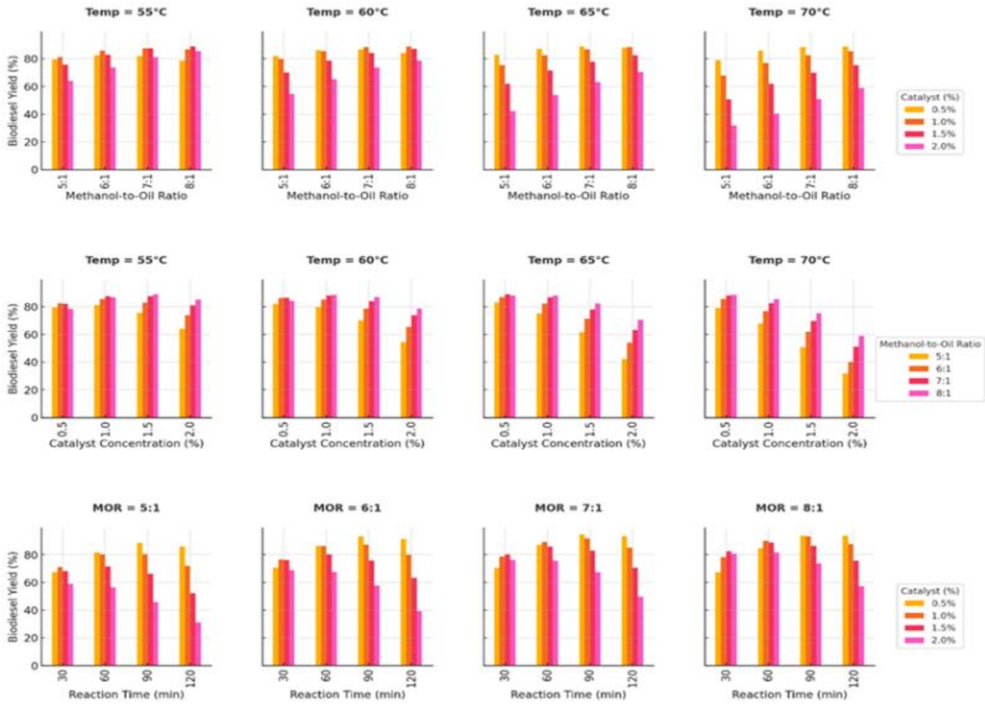

Fig. 3. Influence of methanol-to-oil ratio (MOR), catalyst concentration, reaction temperature, and reaction time on biodiesel conversion efficiency

Predictive model design, validation, and process optimization

To develop robust and accurate predictive models for algal oil extraction and biodiesel conversion, this study integrated three statistical approaches: response surface methodology (RSM), principal component analysis (PCA), and multiple regression analysis (MRA). RSM was employed as the primary optimization technique to evaluate the individual and interactive effects of key process parameters, enabling the identification of optimal operating conditions through a minimal number of experimental runs. PCA was incorporated to reduce data dimensionality, uncover correlations among variables, and highlight the most influential factors affecting process efficiency. This helped in simplifying the model structure and validating the consistency of the RSM results. Meanwhile, MRA was utilized to establish quantitative relationships between the input variables and response outcomes, allowing for the construction of statistically validated regression equations that enhance the predictive power and interpretability of the models.

Predictive model for algal oil yield

The predictive model for algal oil yield was developed using Response Surface Methodology to evaluate the effects of Algal biomass-to-solvent ratio, algal particle size, and contact time on oil extraction efficiency. The regression analysis yielded a quadratic model equation that accurately predicts algal oil yield based on the key process variables:

$$Y = 8.3602 - 4.0342 \times BS - 1.0439 \times APD + 0.4806 \times Time + 4.0249 \times BS^2 - 0.0414 \times Time^2$$

where **Y represents the predicted oil yield (g), BS is the biomass-to-solvent ratio, APD refers to particle size (mm), and Time denotes contact time (h). The high R^2 value (0.993) confirms the model's ability to explain nearly all variations in oil yield, while the low RMSE (0.0397) ensures minimal error in predictions.The model demonstrated exceptional predictive accuracy, with an R^2 value of 0.993, indicating that 99.3% of the variation in oil yield can be attributed to these variables.**

The regression analysis revealed that contact time has a significant positive effect on oil yield (+0.4806), but its quadratic term (-0.0414 for $Time^2$) suggests that after an optimal duration, the increase in yield slows or plateaus. Similarly, particle size negatively affects oil yield (-1.0439), indicating that smaller particle sizes enhance extraction efficiency by increasing the surface area available for solvent interaction.

The biomass-to-solvent ratio showed a nonlinear relationship with oil yield. The trend confirms that increasing the solvent amount relative to biomass improves oil extraction efficiency. However, the quadratic nature of the regression model suggests the existence of an optimal BS ratio beyond which further increases may not significantly enhance oil yield.

Based on the RSM-based predictive model, the optimal conditions for maximizing algal oil extraction efficiency were identified as a biomass-to-solvent ratio (BS) of 1:8, a particle size of 0.355 mm, and a contact time of 4 hours, yielding a predicted oil output of 8.82 g.

Figure 4 illustrates the correlation between actual and predicted oil yield values using the RSM-based predictive model, demonstrating a strong agreement and high model accuracy with minimal deviation from the ideal regression line.

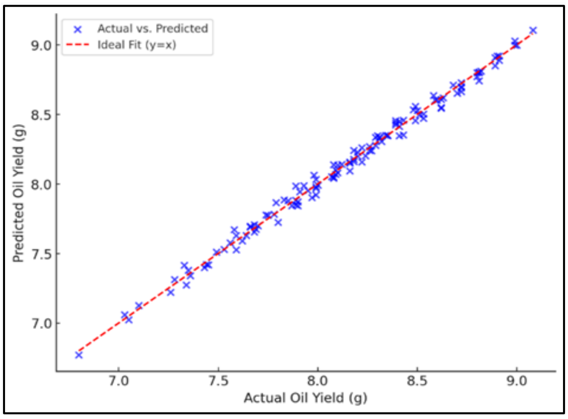

Fig. 4. Correlation between actual and predicted oil yield values using the RSM-based predictive model

Predictive model for Biodiesel yield

The predictive model for biodiesel yield (%) was developed using Response Surface Methodology to assess the impact of four key parameters: Methanol-to-Oil Ratio , Catalyst Concentration, Reaction Temperature , and Reaction Time . A second-order polynomial regression model was developed to analyze both the linear and interaction effects of these parameters, providing a comprehensive understanding of their impact on biodiesel conversion efficiency.The RSM model equation obtained is as follows:

$$Y = 5.525\,X_1 - 8.443\,X_2 - 4.865\,X_3 - 1.018\,X_4 + \beta_{ii}\,X_i^2 + \beta_{ij}\,X_i\,X_j + \text{Intercept}$$

Where Y represents the Biodiesel yield (%), and X_1,X_2,X_3 and X_4 correspond to MOR, catalyst concentration, reaction temperature, and reaction time, respectively. The model coefficients indicate that MOR has a strong positive effect (+5.525) on biodiesel yield, while Catalyst Concentration (-8.443) and Reaction Temperature (-4.865) negatively influence yield when used excessively. Reaction Time has a mild negative impact (-1.018), suggesting that extended reaction durations do not significantly enhance yield and may lead to biodiesel degradation.

The high R² value of 0.994 confirms the model's ability to explain nearly all variations in biodiesel yield, indicating an excellent fit to the experimental data. The low RMSE (1.194) ensures minimal error in predictions, demonstrating the model's robustness in accurately estimating biodiesel yield under different process conditions.

The model results show that increasing MOR significantly improves biodiesel yield due to the enhanced availability of methanol for the transesterification reaction. However, excessive MOR beyond (7:1) does not result in further yield improvement and may increase production costs due to excess methanol recovery.

The strong negative coefficient (-8.443) for catalyst concentration suggests that higher catalyst concentrations beyond 1.5% lead to lower biodiesel yield due to the formation of soap, which reduces biodiesel purity. These findings highlight the importance of maintaining an optimal catalyst concentration (~1.5%) to avoid undesirable side reactions.

The regression coefficient for reaction temperature (-4.865) suggests that excessive heating negatively impacts biodiesel yield. While increasing temperature enhances reaction kinetics and reduces oil viscosity, excessive temperatures above 65°C lead to methanol evaporation, thereby reducing its availability for the reaction.

The mild negative effect (-1.018) for Reaction Time indicates that reaction duration beyond 90 minutes does not significantly improve biodiesel yield. The results confirm that the reaction reaches equilibrium within 90 minutes, beyond which additional reaction time leads to unnecessary energy consumption without significant yield enhancement.

The Response Surface Methodology (RSM)-based predictive model was used to determine the optimal operating conditions for maximizing biodiesel yield while ensuring process efficiency. The model identified the optimal ranges as follows: Methanol-to-Oil Ratio (7:1), Catalyst Concentration (1.5%), Reaction Temperature (65 °C), and Reaction Time (90 min). These values represent the best balance between reactant availability, catalyst effectiveness, and reaction kinetics.

Figure 5 illustrates the correlation between actual and predicted biodiesel yield using the RSM-based predictive model, The correlation plot reveals a strong agreement between actual and predicted biodiesel yield values, confirming the effectiveness of the RSM model in accurately capturing the influence of process parameters.

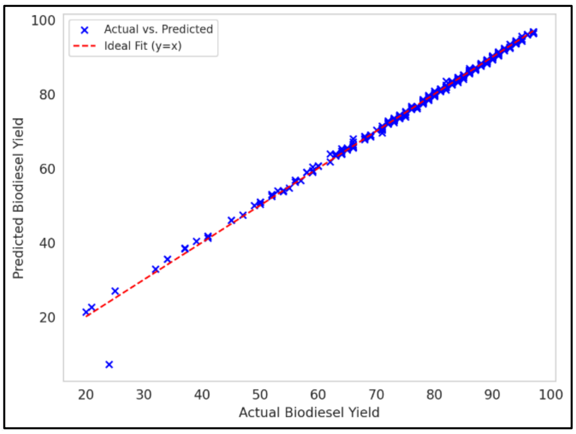

Fig. 5. Correlation between actual and predicted biodiesel yield

The high R² value of 0.994 indicates that 99.4% of the variation in biodiesel yield is explained by the model, demonstrating an excellent fit to the experimental data. Furthermore, the low RMSE (1.194) signifies minimal error in predictions, reinforcing the reliability of the developed regression model..

Properties of produced biodiesel

The purity and quality of the biodiesel produced from algal oil via transesterification were evaluated based on compliance with ASTM D6751 standards. Key parameters including density and kinematic viscosity were measured to assess the fuel's physical characteristics and ensure its suitability as a renewable fuel alternative. Density and kinematic viscosity at 40°C were within standard limits, ensuring proper fuel atomization, efficient combustion, and stable engine operation. The flash point exceeded the minimum requirements, enhancing storage safety and reducing fire risks. The acid number and saponification value complied with maximum limits, ensuring oxidative stability and long-term storage viability. The iodine value, slightly below the maximum threshold, suggests good oxidative. The physicochemical properties of the produced biodiesel, including density, viscosity, and acid number, complied with ASTM D6751 specifications as shown in Table 1.

Table 1. The properties of produced biodiesel compared with international standards

Property	Unit	Produced Biodiesel	ASTM D6751Test Method	ASTM D6751 Limits
Density at 15 °C	kg/m³	882	D4052	860–900
K. viscosity at 40 °C	mm²/s	4.1	D445	1.9–6.0
Flash point (min.)	°C	150	D93	130,Min
Acid number (max.)	mg KOH/g	0.29	D664	0.50,Max
Saponification value	mg KOH/g	190	D1693	170–200
Iodine value	g I_2/100g	118	D6584	120,Max

Environmental and economic implications

The process improvements achieved in this study extend beyond yield optimization to deliver measurable environmental and economic benefits. Reduced solvent and catalyst requirements lead to lower chemical waste and energy demands, contributing to improved environmental sustainability. Moreover, maximizing biodiesel conversion efficiency under optimal conditions reduces raw material losses and processing time, thereby lowering operational costs. These factors together enhance the overall feasibility of microalgae-based biodiesel as a green alternative to fossil fuels.

CONCLUSION

This study successfully optimized algal oil extraction and biodiesel production by systematically investigating key process parameters. For oil extraction, the optimal conditions were identified as a biomass-to-solvent ratio of 1:8, particle size of 0.355 mm, and a contact time of 4 hours, yielding a maximum oil output of 8.82 g. The developed Response Surface Methodology model demonstrated high predictive accuracy (R^2 = 0.993), confirming the strong influence of these parameters on extraction efficiency. Similarly, biodiesel conversion was optimized at a methanol-to-oil ratio of 7:1, catalyst concentration of 1.5%, reaction temperature of 65°C, and reaction time of 90 minutes. The biodiesel model exhibited exceptional accuracy (R^2 = 0.994), validating its reliability for process control. The study further highlighted that excessive catalyst concentrations and prolonged reaction times can lead to undesirable side reactions, negatively impacting biodiesel yield.

Quality assessment confirmed that the biodiesel produced under optimized conditions met ASTM D6751 standards, ensuring its suitability as a renewable fuel. Key properties, including density, viscosity, flash point, acid number, saponification value, and iodine value, were all within acceptable limits, demonstrating favorable oxidative stability. These findings contribute significantly to advancing algal biofuel technology by establishing precise process parameters that enhance efficiency while maintaining product quality.

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تحسين عملية استخلاص الدهون والتحويل الإستري لتعزيز إنتاج الديزل الحيوي من الطحالب الدقيقة كمصدر للطاقة الخضراء

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الملخص

تم إجراء دراسة شاملة بهدف تحسين استخلاص زيت الطحالب وتحويله إلى الديزل الحيوي من خلال التقييم المنهجي للمعاملات التشغيلية الأساسية باستخدام منهجية الاستجابة السطحية (RSM). تم تقييم مرحلة الاستخلاص عبر تغيير نسبة الطحالب إلى المذيب (BS)، وقطر جسيمات الطحالب (APD)، وزمن التلامس أثناء الاستخلاص (ET)، بينما تم تحسين عملية التحويل الإستري من خلال تحليل تأثير نسبة الميثانول إلى الزيت (MOR)، وتركيز الحفاز، ودرجة حرارة التفاعل، وزمن التفاعل. أظهرت التحليلات الإحصائية باستخدام تحليل التباين (ANOVA) وتقنيات الانحدار أن زمن التلامس يعزز بشكل كبير من إنتاجية الزيت ($R^2 = 0.445, p < 0.001$)، في حين أظهر قطر جسيمات الطحالب علاقة عكسية واضحة مع العائد ($R^2 = 0.303, p < 0.001$). بالإضافة إلى ذلك، كان لنسبة الطحالب إلى المذيب (BS) تأثير معتدل على إنتاجية الزيت ($R^2 = 0.214, p < 0.001$). أما في عملية إنتاج الديزل الحيوي، فقد ساهمت كل من نسبة الميثانول إلى الزيت، تركيز الحفاز، درجة حرارة التفاعل، وزمن التفاعل في كفاءة التحويل، حيث أظهرت درجة حرارة التفاعل التأثير الإيجابي الأقوى ($R^2 \approx 0.994, p < 0.001$).تم تطوير نماذج تنبؤية باستخدام منهجية الاستجابة السطحية (RSM)، وحققت قيم R^2 بلغت 0.993 و0.994 لإنتاجية زيت الطحالب والديزل الحيوي على التوالي، مع قيم خطأ متوسط الجذر التربيعي (RMSE) بلغت 0.0397 و1.194 ، مما يؤكد على دقة النماذج التنبؤية وقوتها.