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Strategic Enhancement of Microalgae-Based Biodiesel Production Efficiency through Chemical Acidic Hydrolysis and Advanced Artificial Neural Network Modeling

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ABSTRACT

Biodiesel production from microalgae is often hindered by inefficiencies inherent in traditional optimization methods, which rely heavily on empirical data and frequently fail to achieve optimal efficiency and economic viability. This study introduces a novel approach that integrates Artificial Neural Network (ANN) modelling with experimental optimization to address these challenges, aiming to enhance biodiesel yield, optimize cost-effectiveness, and improve sustainability. Extensive experimentation was conducted to optimize key parameters in the biodiesel production process. Using sulfuric acid (H_2SO_4) as a catalyst, a maximum biodiesel yield of 97.46% was achieved under specific conditions: 2 hours reaction time, 40°C temperature, 0.5 M concentration, 400 RPM stirring speed, a methanol-to-oil ratio of 12:1, and 50 g/L Chlorella vulgaris biomass. The process involved lipid extraction, centrifugation at 3500 RPM for 15 minutes, transesterification at 55°C and 600 RPM for 20 minutes, followed by phase separation. ANN modelling played a crucial role in optimizing these experimental conditions. The Feed Forward ANN model successfully predicted the optimal conditions as 2.175 hours, 86.28°C, 347.15 RPM, and 0.5 M acid concentration, with a projected yield of 99.53%. Implementing these predicted parameters resulted in a biodiesel yield of 98.12%, surpassing the experimental yield and closely aligning with the forecasted yield. These results underscore the precision and effectiveness of ANN modelling in optimizing biodiesel production, significantly enhancing efficiency, reducing costs, and shortening experimental durations. This study represents a significant advancement in sustainable energy solutions, demonstrating the transformative potential of ANN modelling in the efficient production of biodiesel from microalgae.

Keywords: Microalgae; biodiesel production; Artificial Neural Network (ANN) optimization; acidic hydrolysis; transesterification reaction

INTRODUCTION

The demand for sustainable energy is rising due to dwindling fossil fuel reserves and environmental challenges. Research into alternative fuels such as biodiesel, biomethane, and bioethanol is gaining momentum. With their high lipid content and adaptability, microalgae could improve biodiesel synthesis efficiency and profitability (Chuah et al. 2017; Mofijur et al. 2013). Microalgae's diversity includes various photosynthetic organisms that can synthesize valuable organic compounds, making them vital in sustainable biotechnology (Abdullah et al. 2019; Chisti 2007, 2008). Microalgae drive biochemical processes for biofuels, bioproducts, and environmental remediation by utilizing solar energy, carbon dioxide (CO2), and aquatic nutrients.

Their enormous growth potential and efficient resource use make them a promising option for sustainable biofuel production, addressing the rising demand for renewable energy (Cheah et al. 2023).

The complexity of bioenergy processes underscores the need for optimization to achieve the highest biofuel yields (Azizi et al. 2018). (Azizi et al. 2018). Microalgae cell walls are often broken down, releasing key components for extraction using chemical treatments involving moderate to high temperatures and chemicals such as HCl,

H_2SO_4, NaOH, or KOH (Rojo et al. 2023).

Alkaline and acidic methods are employed to hydrolyze triglycerides into fatty acids and glycerol, though challenges like saponification and cost complexities persist (Timira et al. 2022) (Kim et al. 2013).

Biodiesel from algae is mainly produced through transesterification, or alcoholysis, which converts vegetable oil into fatty acid alkyl esters (FAAE) using alcohol, followed by filtration to produce algal biodiesel (Ma & Hanna, n.d.).FAAEs, such as FAME and FAEE, have lower viscosity, greater fluidity, and better low-temperature performance than pure vegetable oil. Ester combustion emits carbon from atmospheric CO_2 plants absorb via photosynthesis, making them carbon neutral. The transesterification process, similar to hydrolysis, replaces glycerin in oil with methanol or ethanol, producing biodiesel and glycerol as a by-product. These sequential, reversible reactions convert triglycerides into diglycerides, monoglycerides, and glycerol. To favor biodiesel production, strategies like using excess alcohol or staging the reaction with intermittent glycerol removal are used to optimize yield and manage equilibrium (Hidekl Fukuda 2001). To optimize biofuel production from microalgae, thorough investigation and modification of key elements including microalgae properties, production conditions, catalyst type and dosage, and reactor configuration are essential.

ANN modeling is utilized for optimization, leveraging machine learning to identify patterns and refine the biodiesel production methodologies (Ebrahimpour et al. 2008; Fatiha et al. 2013), (Kusumo et al. 2017; Ong et al. 2021). This study aims to validate and enhance previous experimental and modeling data using ANN, predicting optimum conditions for biodiesel production from microalgae(Liu et al. 2023).

This research evaluates the sustainability of microalgal biomass extraction over the long term. Chemical catalysts for example HCl, H_2SO_4, KOH, and NaOH break down complex microalgal chemicals into simpler compounds suited for biodiesel production(Megawati et al. 2022). Computational models and simulations using MATLAB software capture the intricate dynamics of the extraction process, refining predictive capabilities and identifying critical parameters influencing extraction efficiency.

Ultimately, this research aims to significantly improve efficiency in biodiesel manufacturing from microalgae, contributing to a more sustainable energy future by reducing reliance on fossil fuels. By optimizing extraction processes, higher yields of proteins and carbohydrates for biodiesel conversion can be achieved, advancing environmentally friendly biofuel production practices and promoting global energy sustainability.

MATERIALS

Organic Chlorella, a green algae species thriving in freshwater environments, this alga belongs to the Chlorophyta classification and represents the earliest form of plant life within the Chlorella genus, characterized by single-cell structures. This versatile species is known to flourish in various habitats, including tropical and temperate freshwater and seawater. Its wide distribution renders it a valuable resource, notably abundant in the Southeast Asian region, where it serves as a primary focus for production. Chemicals such as methanol (99.8%), sodium hydroxide (99.6%), sulfuric acid (96%), and distilled water were sourced from local markets in Malaysia.

METHODOLOGY

ACIDIC HYDROLYSIS USING H_2SO_4 METHOD WITH TRANSESTERIFICATION

Research has focused on optimizing conditions for biodiesel synthesis using microalgae (Rahman et al. 2017) discovered that 1.5% sulfuric acid, 12:1 molar ratio, 60°C, 90 minutes, and 400 rpm mixing optimally esterified Spirulina maxima. Transesterification worked best at 65°C, 9:1 methanol-to-oil ratio, 90 minutes, 600 rpm, and 0.75% catalyst. (Muhammad et al. 2022) optimized Chlorella pyrenoidosa FAME production with hydrochloric acid using ANN and RSM modeling. The optimal conditions were 146°C, 2.83 solvent-to-wet biomass ratio, 3.86M hydrochloric acid, and 240 minutes, though further research was needed due to limitations in parameter range and data validation.

A one-step acidic hydrolysis procedure at 120°C for 180 minutes by (Cao et al. 2013) produced 92.5% biodiesel from Chlorella pyrenoidosa Their study highlighted the need for more detailed experimental validation and sustainability assessments.

A methodical approach was utilized to begin extracting fatty acid methyl esters (FAME) from Chlorella vulgaris as shown in Figure 1. Initially, 25 g of Chlorella vulgaris powder was carefully mixed with 0.5 L of distilled water. This stage was critical since it intended to produce an environment suitable for the extraction procedure. The temperature range of 40°C to 120°C was chosen deliberately since differing temperatures may alter lipid extraction efficiency owing to differential solubility and reaction kinetics.

Furthermore, the concentration of sulfuric acid (H_2SO_4) was varied between 1M and 0.5M. This parameter

was meticulously adjusted to optimize the acidic hydrolysis process, as the acid concentration can influence the breakdown of cell walls and release of lipids from the microalgae biomass. Additionally, mixing speeds ranging from 300 rpm to 400 rpm were explored, as agitation plays a critical role in facilitating mass transfer and enhancing extraction efficiency. Moreover, extraction times of 1, 2, and 4 hours were meticulously selected to assess the impact of prolonged exposure on lipid yield.

Upon completion of the initial lipid extraction step, the mixture was carefully transferred into a centrifuge tube. This transfer ensured the separation of solid biomass from the liquid phase containing the extracted lipids, facilitating downstream processing.

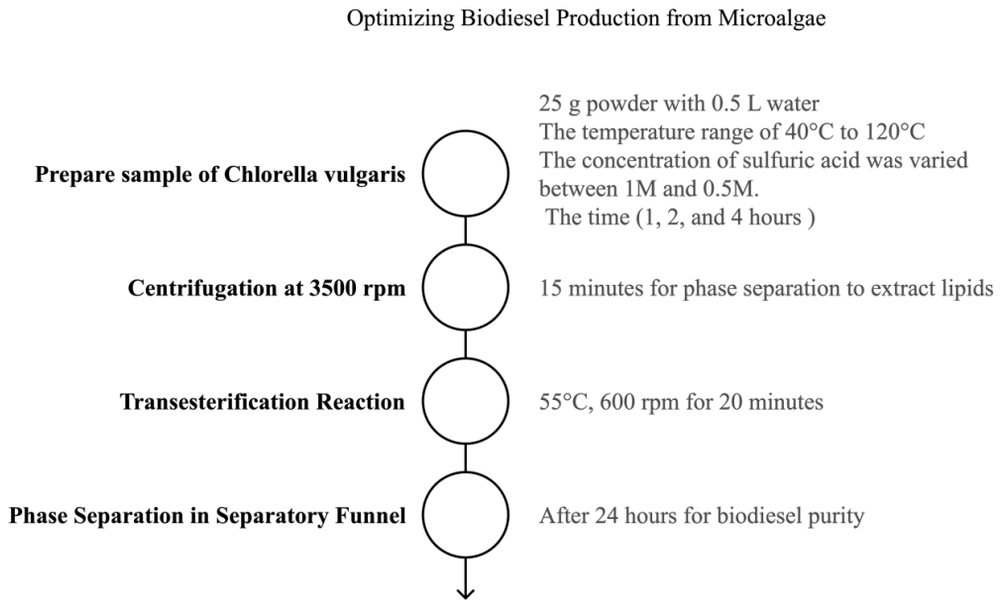

FIGURE 1. Acid hydrolysis and transesterification reaction for biodiesel production from algae.

For the subsequent phase of lipid extraction, the mixture underwent centrifugation at 3500 rpm for 15 minutes. This step was pivotal in achieving phase separation, as centrifugal force effectively separated the lipid phase from the aqueous phase.

Transesterification, a crucial step in biodiesel production, was meticulously executed by combining the extracted lipid solution with methanol and an alkaline catalyst. The choice of methanol-to-oil ratio (12:1) and the reaction conditions (55°C, 600 rpm for 20 minutes) were optimized to ensure efficient conversion of lipids into FAME and glycerol. Lastly, phase separation to separate biodiesel from glycerol was carried out using a separatory funnel after 24 hours. This duration allowed for complete phase separation, ensuring the purity and quality of the biodiesel product.

In summary, each step of the extraction and transesterification process was carefully designed and executed to optimize lipid yield and biodiesel production efficiency.

ANN MODELING OPTIMIZATION

The ANN Feed Forward Modeling technique was employed in this work to cross-reference, compare, evaluate, and analyze data from earlier investigations, which included both modeling and experimental datasets.

Figure 3 illustrates the diagram of an ANN used in this study. The ANN consists of input, hidden, and the output layers. The input layer is where external data is sent into the neural network for analysis or training. The data then passes through one or more hidden layers, which transform the input into useful information for the output layer.

Finally, the ANN's response to the supplied input data is presented as an output by the output layer, as depicted in Figure 2, and Figure 3.

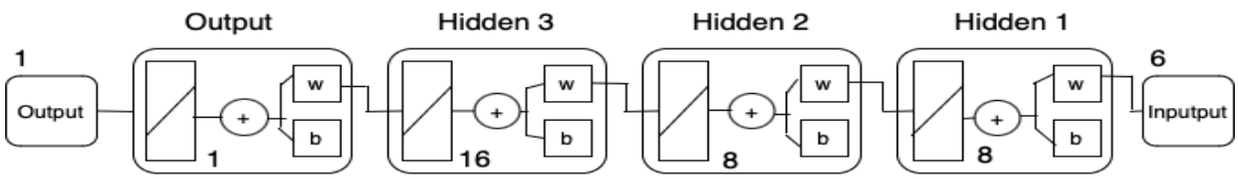

FIGURE 2. The diagram of an Artificial Neural Network (ANN) used in this work.

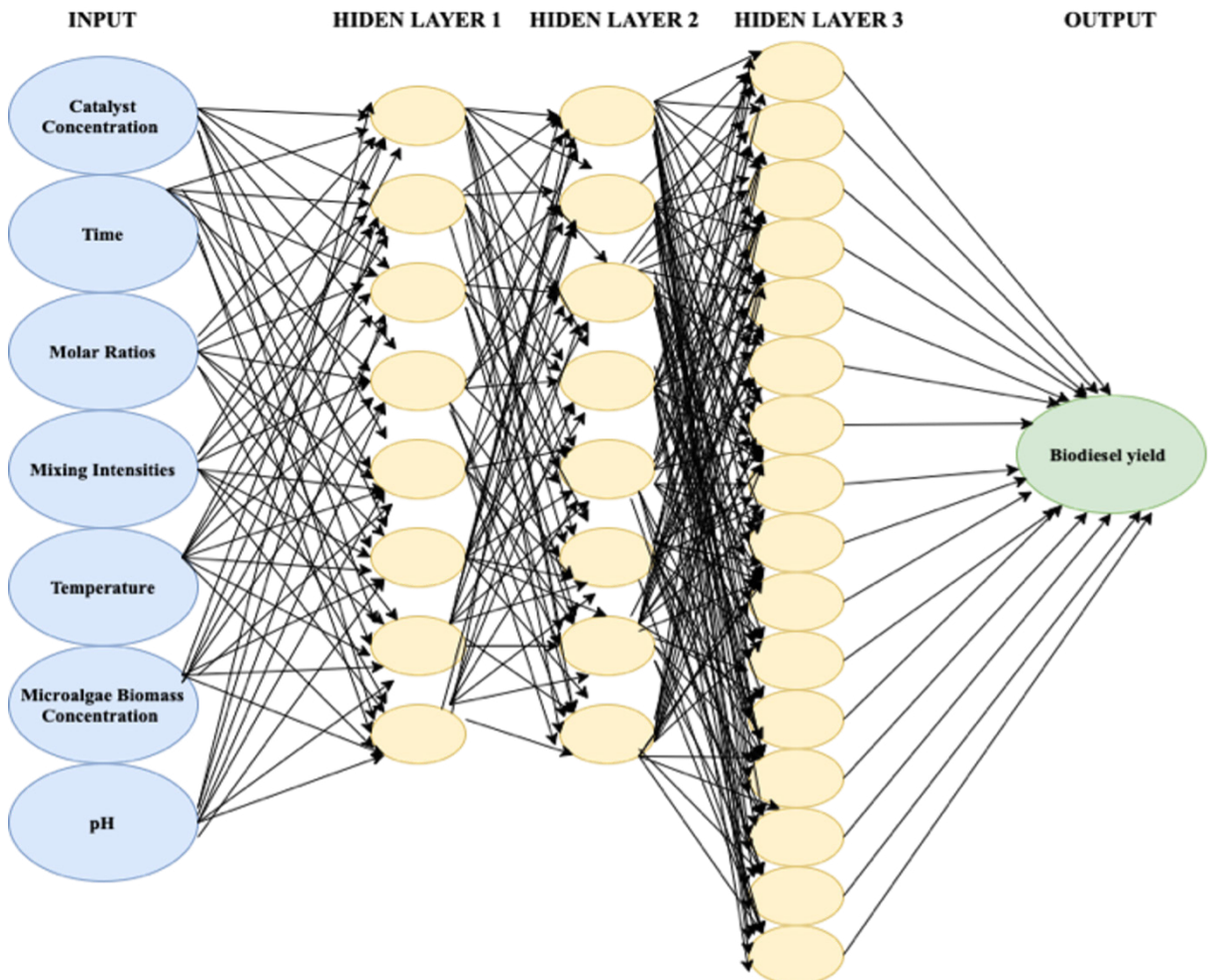

FIGURE 3. The diagram of an Artificial Neural Network (ANN) used in this work

The architectural configuration of the ANN used in this work featured three hidden layers with 8 neurons in the first two layers and 16 neurons in the final layer, utilizing the hyperbolic tangent (tanh) activation function. This configuration, denoted as [8, 8, 16] as shown in Figure 3, strikes a balance between model complexity and computational efficiency, facilitating the hierarchical abstraction of features and capturing non-linear relationships within the data while preventing overfitting.

Weights (w) and biases (b) in the neural network were randomly initialized and iteratively adjusted using backpropagation and the Levenberg-Marquardt (LM) optimization method. The error between predicted outputs ($y^\wedge$) and actual outputs (y) was calculated using the loss function (Svozil et al. 1997):

$$\text{Loss} = \frac{1}{2}(y - y^\wedge)^2. \quad (1)$$

The equations for a feed-forward neural network could be represented as follows for the hidden layers:

The output of the first hidden layer, $h_{1,j}$, is calculated as (Svozil et al. 1997):

$$h_{1,j} = f(\sum_{k=1}^{8} w_{1,k,j} . x_k + b_{1,j}) \quad (2)$$

Subsequently, the output of the second hidden layer, $h_{2,j}$, is computed as:

$$h_{2,j} = f(\sum_{k=1}^{8} w_{2,k,j} . h_{1,k} + b_{2,j}) \quad (3)$$

The output of the second hidden layer, $h_{3,j}$, is computed as:

$$h_{3,j} = f(\sum_{k=1}^{16} w_{3,k,j} . h_{2,k} + b_{3,j}) \quad (4)$$

For the output layer, let y represent the output of the neural network. The equation is (Svozil et al. 1997):

$$y = \sum_{k=1}^{16} w_{4,k} \cdot h_{3,k} + b_4 \quad (5)$$

The performance of the ANN model was evaluated using R-squared (*R*2) values, which measure the accuracy of the predictions:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (Predi - Expi)^2}{\sum_{i=1}^{n} (Predi - \overline{Exp})^2} \quad (6)$$

A high *R*2 value (greater than 90%) indicated strong predictive capabilities. The model was trained on 80% of the data and validated on the remaining 20%, optimizing parameters like temperature, concentration, mixing speed, and time to maximize yields. Predictions were experimentally validated in the laboratory to confirm the optimal achievable yield. Additionally, the use of evolutionary optimization strategies to iteratively refine model parameters based on their impact on yield was suggested, combining the interpretability of LM with the optimization prowess of evolutionary algorithms. This systematic approach enabled a thorough analysis and accurate prediction of biodiesel yields, leveraging the power of ANN for complex data interpretation and validation.

RESULTS

CHEMICAL HYDROLYSIS RESULTS

ACIDIC HYDROLYSIS USING H_2SO_4

The research investigated acidic hydrolysis using H_2SO_4 at varying concentrations of 0.5 M and 1 M as catalysts, alongside 50 g/L of algae powder from Chlorella vulgaris and a methanol-to-oil ratio of 12:1. These studies were carried out under various conditions, including the temperatures of 40°C, 60°C, and 120°C, mixing rates of 300 RPM and 400 RPM, and reaction periods of 1 hour, 2 hours, and 4 hours. These trials' findings, which are laid out in a table, provide vital information on how these characteristics affect how efficiently biodiesel is produced. The present thorough research serves as the foundation for assessing the ideal conditions for acidic hydrolysis in the manufacture of biodiesel, therefore making a substantial contribution to the progress of sustainable energy technologies.

METHANOL TO OIL RATIO

The methanol-to-algal oil ratio is pivotal in transesterification efficiency. While the stoichiometric ratio is 3:1, higher ratios, such as 12:1, ensure thorough conversion, overcoming kinetic barriers and enhancing overall efficiency. Research and ANN modeling validate this optimal ratio, showing a positive correlation with biodiesel production. This ratio was tested across various conditions, indicating its robustness and performance under different scenarios.

CATALYST CONCENTRATION

The investigation explored how catalyst concentration and mixing speed influence biodiesel yield in acidic hydrolysis processes using H_2SO_4 as a catalyst. Ranging from 0.5 to 1 Molar, catalyst concentration and mixing speed between 300 and 400 RPM were examined. Initial parameters included a 40°C temperature, 300 RPM stirring speed, and a 12:1 methanol-to-algal oil molar ratio. Experiments, spanning 1, 2, and 4 hours, scrutinized the evolving effect of catalyst concentration on biodiesel yield. At 0.5 Molar acid concentration, biodiesel yield varied from 61.0224% to 95.8078%, peaking at 120°C temperature, 4 hours reaction time, and 300 RPM mixing speed as shown in

Figure 6.

Mixing speed variations minimally impacted yield, highlighting acid concentration, temperature, and reaction time as pivotal factors. Elevating the mixing speed from 300 to 400 RPM notably enhanced yield, reaching 97.4606% under specific conditions as shown in Figure 4. Increasing H_2SO_4 concentration from 0.5 to 1 Molar significantly boosted biodiesel yield from 12.88% to 95.482% across varying temperatures and durations, maintaining a constant mixing speed of 300 RPM as shown in Figure 5. Under 1 Molar H_2SO_4 and 60°C temperature, 300 RPM and 4 hours duration yielded the highest, whereas, at 400 RPM and 2 hours duration, a comparable yield was attained as shown in Figure 7.

In essence, optimal conditions for maximum biodiesel yield centered on 0.5 Molar acid concentration, with additional benefits observed at increased mixing speeds. These findings stress the necessity of fine-tuning acid concentration and mixing speed for maximal biodiesel production efficiency and yield in acidic hydrolysis, guiding strategies for optimization and cost reduction. Further exploration across parameters is warranted for refining conditions in acidic hydrolysis processes.

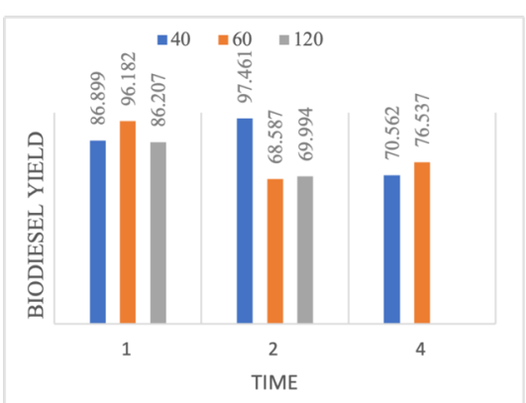

FIGURE 4. Relationship Between Biodiesel Yield and Time At 400 RPM and 0.5M At Different Temperature.

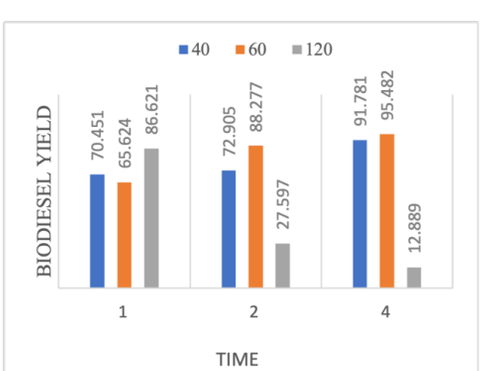

FIGURE 5. Relationship Between Biodiesel Yield and Time at 300 RPM and 1M At Different Temperature

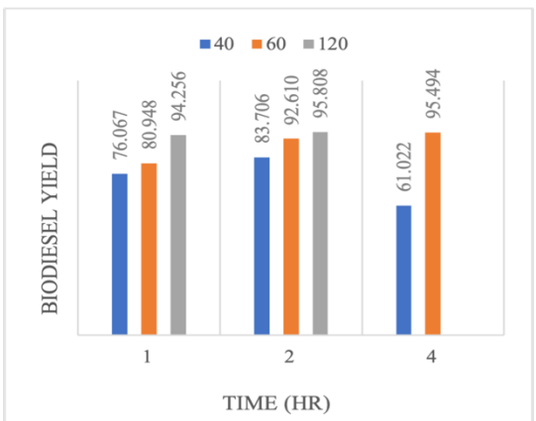

FIGURE 6. Relationship Between Biodiesel Yield and Time At 300 RPM and 0.5 M At Different Temperature.

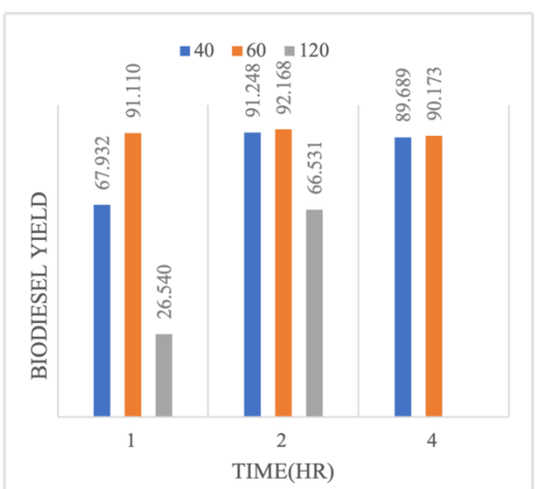

FIGURE 7. Relationship Between Biodiesel Yield and Time At 400 RPM and 1M At Different Temperature.

TEMPERATURE

The experimental investigation analyzed temperature variations' influence on biodiesel production, maintaining controlled parameters. Temperature ranged from 40°C to 120°C, alongside fixed conditions: a 12:1 methanol-to-oil ratio, 50 g/l biomass content, and two catalyst concentrations (0.5 and 1 Molar). Mixing speeds of 300 and 400 rpm were explored over 1, 2, and 4-hour intervals, employing a factorial design for systematic evaluation. At 40°C with 0.5 Molar acid concentration and 300 RPM mixing speed, biodiesel yield varied across time intervals, peaking at 83.706% after 2 hours as shown in Figure 8. Increasing the mixing speed to 400 RPM consistently enhanced yield, with a maximum of 97.461% at 2 hours as shown in Figure 9. Elevating H_2SO_4 concentration from 0.5 to 1 Molar improved yield, for instance, at 40°C and 1 Molar, yields ranged from 70.451% to 91.781% over different durations

as shown in Figure 8. Optimal conditions were observed at 40°C, 0.5 Molar acid concentration, and 400 RPM, yielding 97.461% as shown in Figure 9. Transitioning to 60°C as shown in Figures 10 and 11 increased yield significantly, reaching 96.182% at 400 RPM and 0.5 Molar concentration after 1 hour. At 120°C, yield declined due to increased acid concentration, emphasizing the detrimental effects of excessive heat and higher acid concentrations as shown in Figures 12 and 13. The optimal temperature range for maximum efficiency was observed around 40°C, emphasizing the critical role of temperature in optimizing biodiesel production. These findings provide valuable insights into temperature's nuanced influence on biodiesel synthesis, guiding future research and practical applications for sustainable energy production.

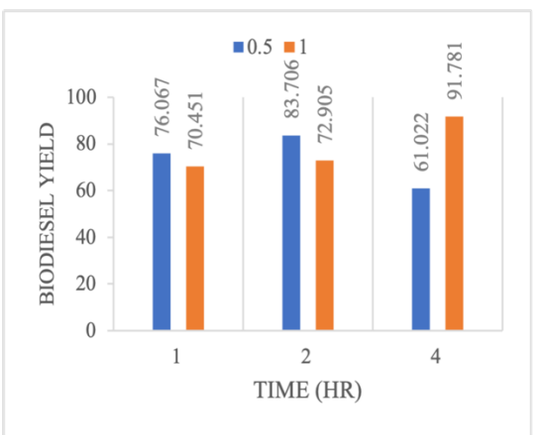

FIGURE 8. The effect of Temperature 40 °C at 300 RPM on Biodiesel Yield With Different Time

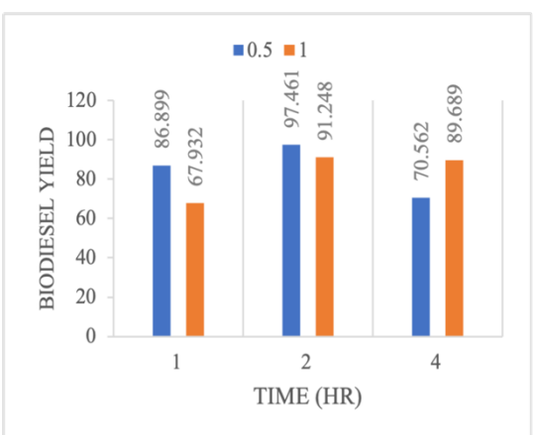

FIGURE 9. The effect of Temperature 40 °C at 400 RPM on Biodiesel Yield With Different Time

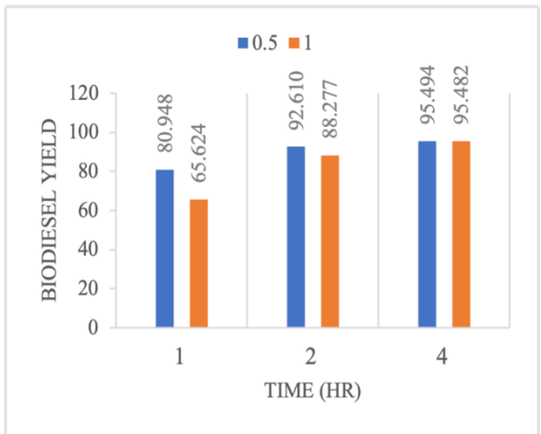

FIGURE 10. The effect of Temperature 60 °C at 300 RPM on Biodiesel Yield With Different Time

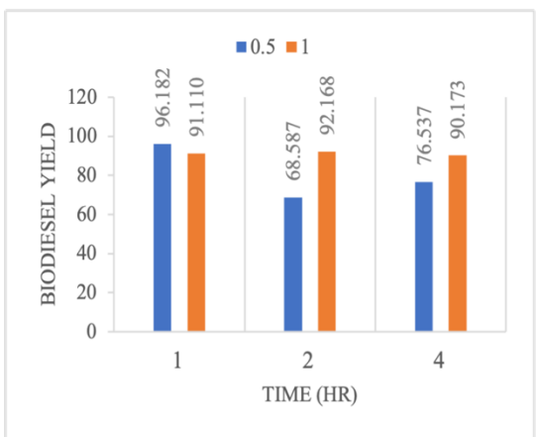

FIGURE 11. The effect of Temperature 60 °C at 400 RPM on Biodiesel Yield With Different Time.

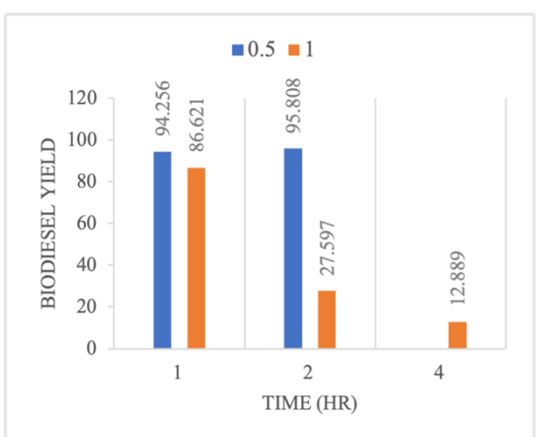

FIGURE 12. The effect of Temperature 120 °C at 300 RPM on Biodiesel Yield With Different Time.

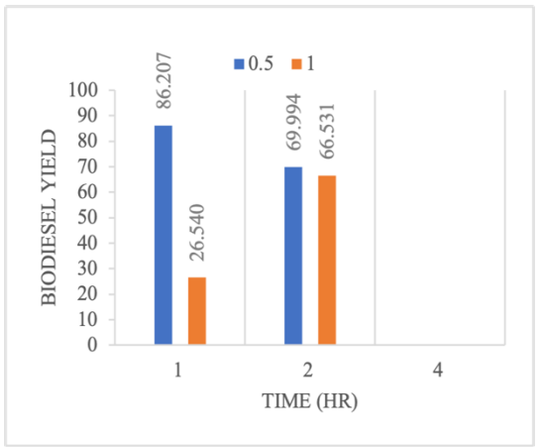

FIGURE 13. The effect of Temperature 120 °C at 400 RPM on Biodiesel Yield With Different Time

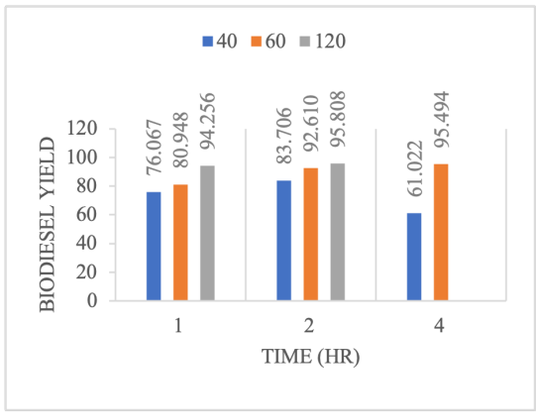

FIGURE 14. Relationship Between Biodiesel Yield And Time at 0.5M and 300 RPM

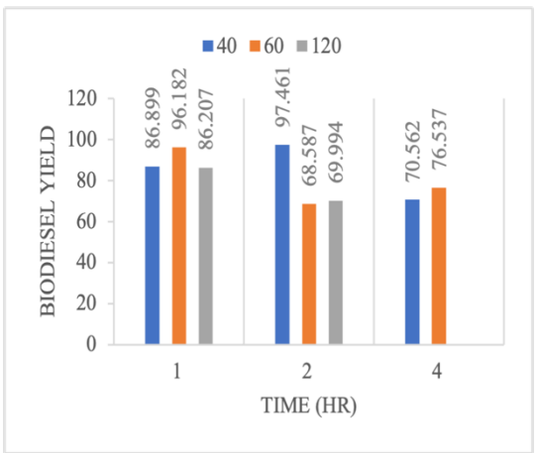

FIGURE 15. Relationship Between Biodiesel Yield And Time at 0.5M and 400 RPM With Different Temperature

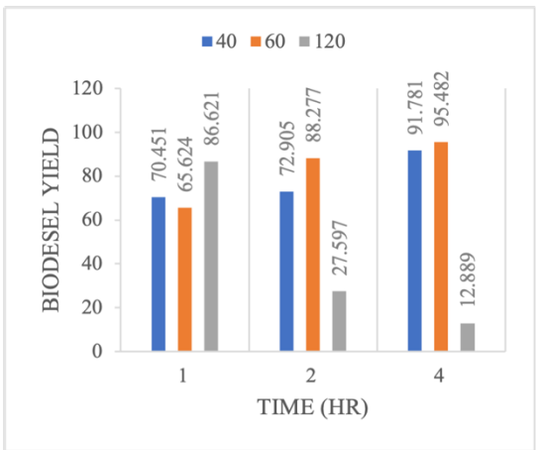

FIGURE 16. Relationship Between Biodiesel Yield And Time at 1M and 300 RPM With Different Temperature

TIME

The investigation explored the influence of reaction time, catalyst concentration, and mixing speed on biodiesel yield under controlled conditions. Initial parameters included a temperature range from 40°C to 120°C, mixing speeds of 300 and 400 RPM, and H_2SO_4 concentrations of 0.5 and 1 Molar, with a constant methanol-to-oil ratio of 12:1 and algae biomass concentration of 50g/L. At 40°C and 0.5 Molar H_2SO_4 concentration, biodiesel yield ranged from 76.067% to 61.022% over 1 to 4 hours, with the highest yield observed at 2 hours. Increasing the mixing speed to 400 RPM improved yield to 97.461% at 2 hours, albeit with diminishing returns over an extended time as shown in Figures 14,15. Raising the temperature to 60°C resulted in peak yields of 95.494% at 4 hours with 0.5 Molar H_2SO_4 and 92.168% at 2 hours with 1 Molar as shown in Figure 17. At 120°C, yields peaked at 95.808% with 0.5 Molar as shown in Figure 14, and 27.597% with 1 Molar at 2 hours as shown in Figure 16. Additionally, increasing H_2SO_4 concentration enhanced yields, with 91.781% achieved at 4 hours with 1 Molar compared to 70.451% at 1 hour with 0.5 Molar as shown in Figure 16. Changing mixing speed also influenced yield, with 97.461% at 2 hours at 400 RPM compared to 70.562% at 4 hours at 300 RPM as shown in Figure 15. At 60°C, increasing mixing speed led to increased yield over time, while at 120°C, yield decreased significantly due to water evaporation as shown in Figures 16 and 17. In summary, the optimal reaction time for maximum biodiesel yield appears to be approximately 2 hours under the specified conditions. These findings underscore complex interactions involved in biodiesel production and highlight the importance of optimizing parameters to maximize yield while ensuring process sustainability and efficiency.

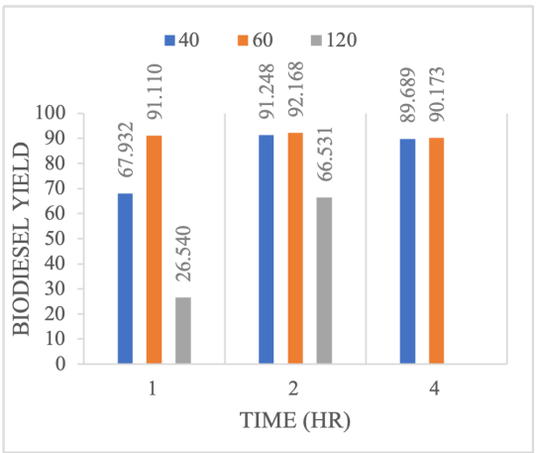

FIGURE 17. Relationship Between Biodiesel Yield And Time at 1M and 400 RPM With Different Tempera

MIXING SPEED

A comprehensive study evaluated the impact of stirring speed on biodiesel production, focusing on an optimal speed of 400 rpm. Under controlled conditions (40°C, 12:1 methanol-to-algal oil ratio, 0.5 M H_2SO_4), stirring speeds of 300 rpm and 400 rpm were tested over 1, 2, and 4 hours. At 40°C, yields improved from 76.067% at 300 rpm to 86.899% at 400 rpm after 1 hour, reaching 97.461% at 400 rpm after 2 hours, and stabilizing at 70.562% after 4 hours. Changing the acid concentration to 1 M showed varied results. At 300 rpm, the yield decreased from 70.451% to 67.932% after 1 hour, rose to 91.248% after 2 hours, and slightly declined to 89.689% after 4 hours. At 400 rpm, a similar diminishing pattern was observed as shown in Figures 18 and 19. At 60°C, yields at 300 rpm increased from 80.948% to 96.182% after 1 hour but declined to 76.537% after 4 hours. With 1 M acid, yields at 300 rpm rose from 65.624% to 91.11% after 1 hour and stabilized at 90.173% after 4 hours as shown in Figures 20 and 21. At 120°C, yields declined at both speeds due to water evaporation as shown in Figures 22 and 23.

These findings underscore the importance of optimizing stirring speed, temperature, and acid concentration, with 400 rpm identified as the optimal stirring speed for maximizing biodiesel yield.

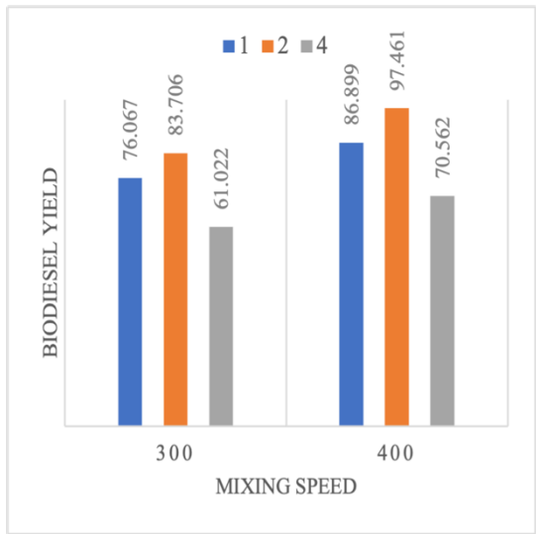

FIGURE 18. Relationship Between Biodiesel Yield And Mixing Speed at 0.5M and 40°C With Different Time.

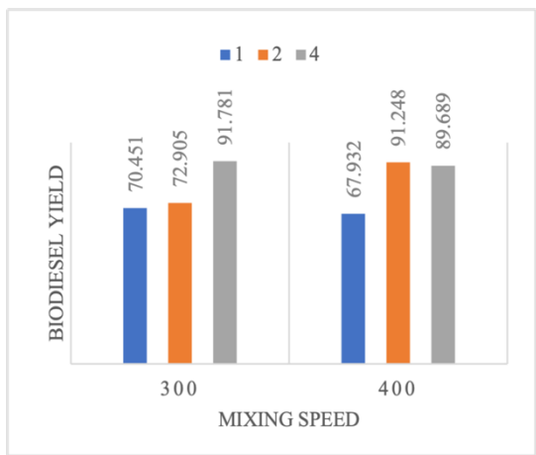

FIGURE 19. Relationship Between Biodiesel Yield And Mixing Speed at 1M and 40°C With Different Time

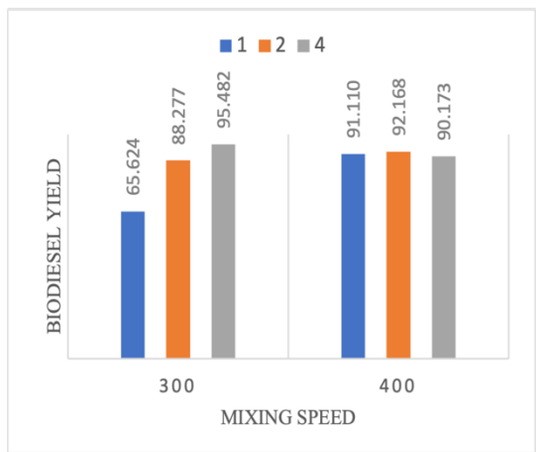

FIGURE 20. Relationship Between Biodiesel Yield And Mixing Speed at 1M and 60°C With Different Time.

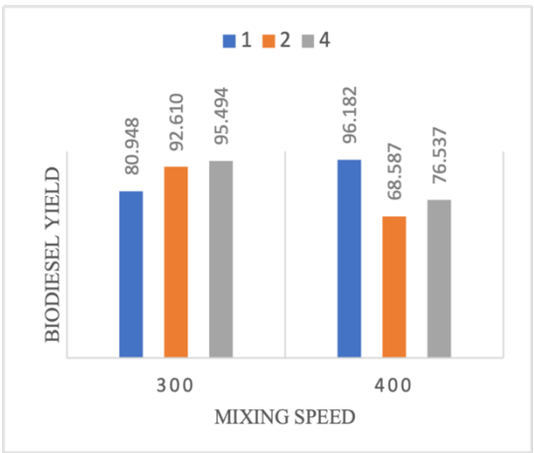

FIGURE 21. Relationship Between Biodiesel Yield And Mixing Speed at 0.5M and 60°C With Different Time

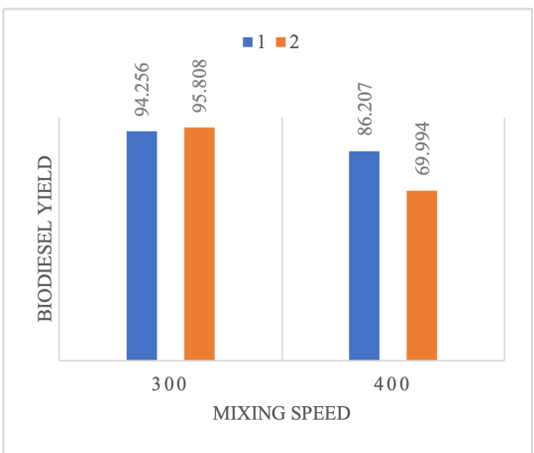

FIGURE 22. Relationship Between Biodiesel Yield And Mixing Speed at 0.5M and 120°C With Different Time.

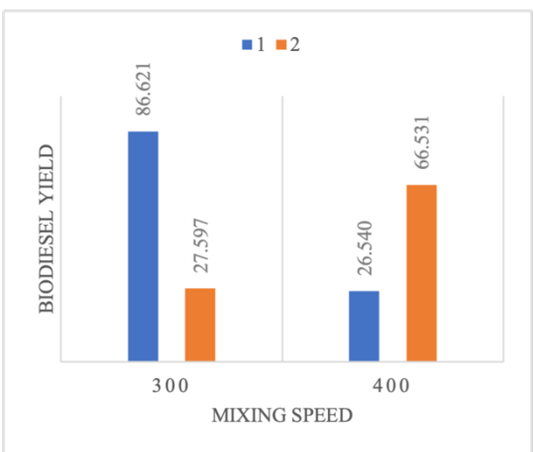

FIGURE 23. Relationship Between Biodiesel Yield And Mixing Speed at 1M and 120°C With Different Time.

CHEMICAL HYDROLYSIS METHOD OPTIMIZATION USING ANN MODELING

The effective hydrolysis of biomass into appealing bioproducts involves an exhaustive and scientific methodology. Acidic hydrolysis, a technique predicated on the use of acids to degrade biomass, emerges as particularly noteworthy due to its capacity to yield optimal quantities of fermentable sugars. This study delves into a meticulous examination of diverse strategies aimed at augmenting the efficacy of acidic hydrolysis. Conversely, alkaline hydrolysis, leveraging alkaline substances to cleave ester bonds, assumes significance in realms such as waste management and biodiesel synthesis. A thorough analysis of existing literature unveils concerted efforts geared towards enhancing the efficiency of alkaline hydrolysis through nuanced adjustments in reaction parameters and agent concentrations. Both acidic and alkaline hydrolysis methodologies play pivotal roles in propelling forward bioenergy production and fostering sustainable practices in biomass utilization.

ACIDIC HYDROLYSIS USING H_2SO_4 METHOD OPTIMIZATION USING ANN

The investigation is initiated by investigating the effect of sulfuric acid concentration (H_2SO_4) on acidic hydrolysis efficiency. The investigation includes doses ranging from 0.5 M to 1 M. Experimental conditions are thoroughly regulated, with a temperature range established at 40°C to 120°C, a methanol to oil ratio of 12:1, and a mixing intensity of 300 to 400 rpm for multiple duration (1 hour, 2 hours, 4 hours). The results indicate the most efficient acidic hydrolysis occurs at an H_2SO_4 concentration of 0.5 M (Table 3). Moving forward, the examination extends to the influence of the molar ratio on the esterification step. Utilizing a molar ratio of 12:1, which aligns with a validated optimum parameter from prior research, the study maintains the temperature at 40°C while keeping other initial conditions constant. It emerges that a molar ratio of 12:1 yields optimal esterification outcomes, as demonstrated in Table 3.

The results unveil that a temperature of 40°C stands out as the most favorable for the esterification process, as illustrated in Table 4. Further exploration involves scrutinizing the impact of time and mixing intensity while maintaining a methanol-to-oil ratio of 12:1, sulfuric acid concentration of 1 M, and temperature of 40°C. Time is varied between 1 hour and 4 hours while mixing intensity ranges from 300 to 400 rpm. Through this meticulous experimental investigation, it is determined that the optimal esterification parameters consist of a time of 2 hours and a mixing intensity of 400 rpm, elucidated in Table 4.

MATLAB software is used to generate an ANN model that is used for verifying the results The model additionally substantially aligns with ideal values determined from previous studies, but it also has an extraordinary coefficient of determination (R2) of 0.94. The optimized parameters furnished by the ANN model closely mirror those obtained from earlier studies, underlining its accuracy and consistency. These outcomes collectively underscore the potential of ANN modeling in enhancing biodiesel manufacturing processes, thereby contributing to heightened efficiency and productivity. The research also highlights the effectiveness of the two-step method for producing biodiesel from chlorella vulgaris providing insightful information for future improvements in biodiesel production methods.

TABLE 1.Optimum Conditions and Artificial Neural Network (ANN) Prediction in Comparison with Experimental Results

Optimal Condition ANN Prediction	Experiment	ANN
Optimal Time (hr)	2	2.175
Optimal Temperature (°C)	40	86.287
Optimal Methanol to Oil Ratio	12:1	12:1
Optimal Stirring Speed (RPM)	400	347.15
Optimal Concentration (M)	0.5	0.5
Biodiesel yield (%)	97.461	99.5315
Biodiesel yield ANN experimentally (%).	98.1249	

Table 1 provides a comprehensive comparison between experimental findings and predictions generated by an artificial neural network (ANN) model for key parameters governing biodiesel production optimization. Firstly, focusing on the optimal time required for the esterification process, both experimental observations and ANN predictions converge remarkably around the 2-hour mark as shown in figure 26 elaborated in table 4. This alignment underscores the robustness and reliability of both the experimental methodology and the predictive capabilities of the ANN model in determining the ideal duration for this critical phase of the process as shown in figure 24 elaborated in table 2. Moving on to temperature optimization, a notable disparity emerges between the experimental setting, which was a 40°C, and the considerably higher ANN-predicted temperature of 86.287°C as shown in figure 25 elaborated in table 3.

The agreement in optimal methanol-to-oil ratio between experimental and ANN-predicted values, both indicating a ratio of 12:1, signifies a consensus on the appropriate stoichiometry for maximizing biodiesel yield. This coherence lends further credence to the reliability of both experimental data and ANN predictions in guiding parameter optimization efforts. However, a slight variance is observed in the optimal stirring speed, with the experimental result indicating 400 rpm while the ANN prediction suggests a slightly lower value of 347.15 rpm as shown in figure 28 elaborated at table 6. Regarding optimal sulfuric acid concentration, both experimental and ANN-predicted values converge precisely at 0.5 M as shown in figure 27 elaborated in table 5, affirming the critical role of acid concentration in catalyzing the hydrolysis process. This alignment underscores the consistency and accuracy of both experimental methodology and ANN modeling in identifying optimal conditions for maximizing biodiesel yield.

Finally, the disparity in biodiesel yield percentage is noteworthy, with the ANN prediction notably higher (99.5315%) compared to the experimental result (97.461%). While the experimental yield remains substantial, Laboratory validation of ANN-predicted parameters confirmed the model's robustness, with experimental yields closely aligning with predictions: H_2SO_4 yielded 98.12%. this variance underscores the potential of the ANN model to predict outcomes with greater precision or identify areas for further optimization while highlighting the complementary roles of experimental data and predictive modeling in refining biodiesel production processes. In conclusion, while there is generally good agreement between experimental results and ANN predictions for most parameters, discrepancies in temperature and stirring speed warrant further investigation and refinement. These findings underscore the utility of ANN modeling in optimizing biodiesel production processes and suggest avenues for enhancing efficiency and yield through synergistic integration of experimental data and predictive modeling insights.

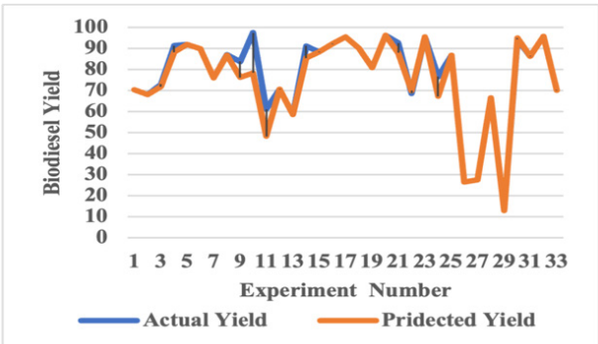

FIGURE 24. The Relationship Between The Actual Experimental Biodiesel Yield Result And Prediction Result Using ANN.

TABLE 2. Actual and Predicted Yield Using ANN

Exp #	ACTUAL YIELD %	PREDICTED YIELD %
1	70.4506	70.4334
4	91.2483	88.2036
8	86.899	86.8096
12	70.5624	70.6108
16	92.1677	92.0267
20	96.1819	96.1857
24	76.5367	67.1533
28	66.5307	66.5249
32	95.8078	95.8145

TABLE 3. Effect of Acid (H_2SO_4) on Biodiesel Yield - ANN Predictions

TEMPERATURE (°C)	ANN BIODIESEL YIELD %
40	59.825
60	96.311
120	81.391

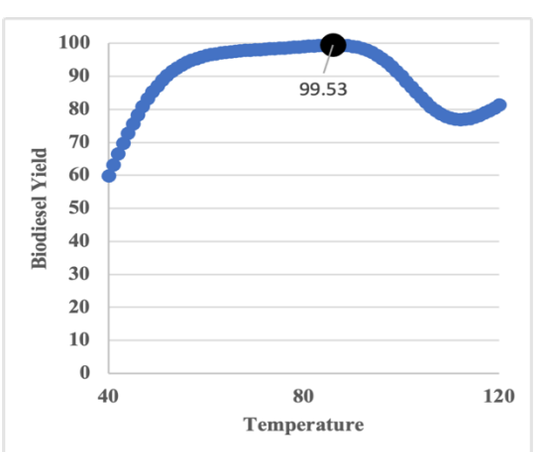

FIGURE 25. The Relationship Between The Prediction Biodiesel Yield Result Using ANN Modelling and the temperature with optimum value

TABLE 4. Effect of Time on Biodiesel Yield – ANN Predictions

TIME (MINUTES)	ANN PREDICTED BIODIESEL YIELD %
60	97.76
120	99.287
180	92.743
240	76.522

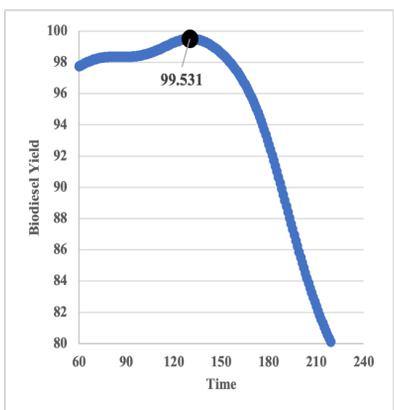

FIGURE 26. The Relationship Between The Prediction Biodiesel Yield Result ANN Modelling And The Time With Optimum Value.

TABLE 4. Effect of Acid Concentration (H□SO□) on Biodiesel Yield - ANN Predictions

H_2SO_4 CONCENTRATION (M)	ANN BIODIESEL YIELD %
0.5	99.532
0.75	96.814
1	55.421

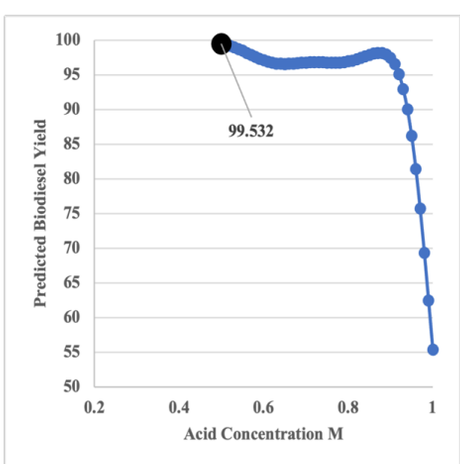

FIGURE 27. The relationship between prediction biodiesel yield result using artificial neural networks ANN modelling using MATLAB and concentration with optimum value.

TABLE. 5 Effect of Acid Stirring Speed (H_2SO_4) on Biodiesel Yield - ANN Predictions

STIRRING SPEED (RPM)	ANN BIODIESEL YIELD %
300	95.037
350	99.516
400	71.524

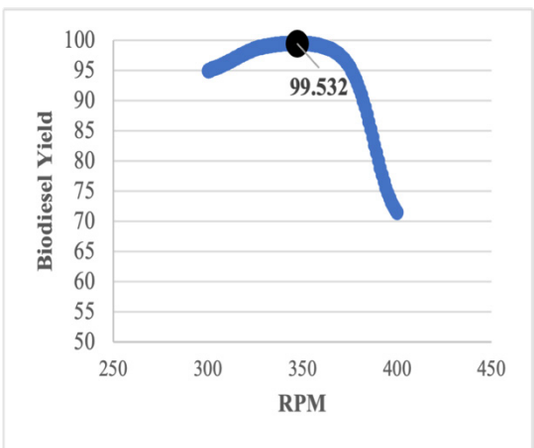

FIGURE 28. The relationship between prediction biodiesel yield result using ANN and stirring speed with optimum value.

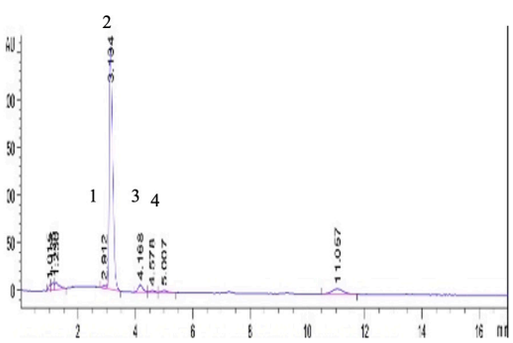

FIGURE 29. HPLC Analysis of the standard mixture of fatty acid derivatized: 1) Linolenic acid (C18:3), 2) Myristic acid (C14), (3: Linoleic acid (C18:2), 4: Palmitic acid (C16).

HPLC analysis of fatty acids utilizes a C18 reversed-phase column, which provides excellent separation efficiency due to its hydrophobic interactions with the fatty acids. The mobile phase typically consists of a gradient or isocratic mix of acetonitrile and water, chosen to optimize the separation of various fatty acid components based on their hydrophobicity. The column is maintained at a temperature between 25-30°C to ensure reproducibility and stable retention times. A flow rate of 1.0 mL/min is standard for achieving optimal resolution within a reasonable analysis time. Injection volumes range from 10-20 µL, allowing for precise quantification without overloading the column. Detection is performed at 210 nm, a wavelength selected to maximize sensitivity for the detection of fatty acid derivatives. This setup is designed to achieve well-resolved peaks and accurate quantification, which is essential for analyzing complex mixtures of fatty acids.

TABLE. 6 Experimental Analysis For FAME Using HPLC At 1M H_2SO_4

Sample	Linolenic acid (C18:3)	Myristic acid (C14)	Linoleic acid (C18:2)	Palmitic acid (C16)	Total FAME%
A1	0.043	0.00	0.00	0.21	0.257
A3	0.021	0.00	0.00	0.89	0.915
A5	0.022	0.00	0.14	1.02	1.174
A2	0.023	0.00	0.14	0.59	0.750
A4	0.022	0.00	0.00	1.07	1.092
A6	0.015	0.00	0.17	1.55	1.740
A7	0.030	0.00	0.00	0.06	0.093
A9	0.009	0.00	0.00	0.24	0.248
A11	0.016	0.00	0.00	0.56	0.572
A8	0.010	0.00	0.14	3.17	3.329
A10	0.017	0.00	0.16	3.18	3.356
A12	0.012	0.00	0.14	3.24	3.384

TABLE 7. Experimental Analysis For FAME Using HPLC At 0.5M H_2SO_4

Sample	Linolenic acid (C18:3)	Myristic acid (C14)	Linoleic acid (C18:2)	Palmitic acid (C16)	Total FAME%
B1	0.0004	8.19	0.102	0.026	8.317
B3	0.0016	7.96	0.102	0.381	8.447
B5	0.0021	8.06	0.000	0.480	8.542
B2	0.0282	0.17	0.000	0.000	0.199
B4	0.0004	6.29	0.182	0.961	7.431
B6	0.0051	8.91	0.000	0.094	11.579
B7	0.0363	0.17	0.000	0.061	0.268
B9	0.0300	0.88	0.000	0.167	1.076
B11	0.0331	0.67	0.110	0.309	1.127
B8	0.0284	0.17	0.126	0.030	0.355
B10	0.0331	0.67	0.110	0.309	1.127
B12	0.0311	1.34	0.000	0.151	1.521

This study examines the impact of sulfuric acid concentrations (0.5 M and 1 M H_2SO_4) on the fatty acid methyl ester (FAME) profiles, which include linolenic acid (C18:3), myristic acid (C14), linoleic acid (C18:2), and palmitic acid (C16). Results indicate that linolenic acid and linoleic acid levels were slightly higher in Set B (0.5 M H_2SO_4), suggesting milder conditions preserve these acids better. Myristic acid was significantly higher in Set B, reaching up to 8.19%, and palmitic acid levels were also higher, reaching up to 3.18%, indicating more effective hydrolysis as shown and elaborated in Tables 7 and 8.

Overall, both sets achieved high total FAME content under optimal conditions. The HPLC results show promising profiles for algal biodiesel, with minimal linolenic acid ensuring stability, significant myristic acid improving cold flow, linoleic acid enhancing oxidative stability and combustion, and palmitic acid balancing fluidity and stability. Higher total FAME in ANN indicates efficient conversion and high purity, supporting algal biodiesel as a viable alternative to fossil fuels.

THE OPTIMUM EXPERIMENT AND ANN

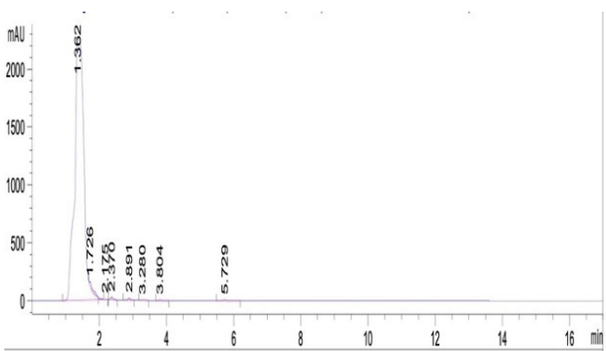

FIGURE 30. HPLC analysis for optimum value through experiment

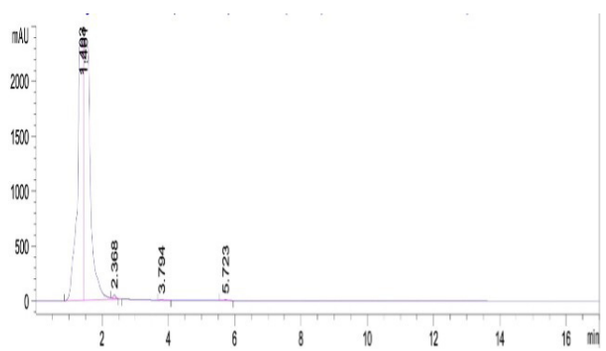

FIGURE 31. HPLC analysis for optimum value using ANN modeling

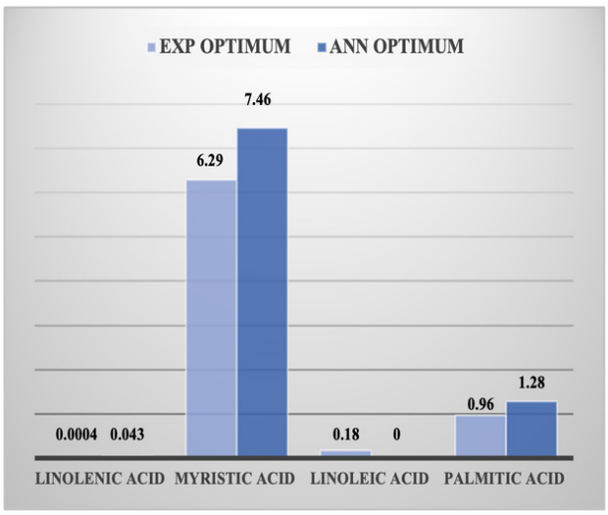

FIGURE 32. Optimum values using experimental and ANN modeling

TABLE 8. Optimum Values Experimental and Using ANN Modeling

Fatty Acid	ANN Value (%)	Experimental Value (%)
Linolenic acid	0.0433	0.0004
Myristic acid	7.46	6.29
Linoleic acid	0	0.18
Palmitic acid	1.28	0.96
Total FAME	8.78	7.431

The comparison of fatty acid composition through test shown in figures 30,31 between laboratory experimental values and experimental predicted ANN values that were implemented in the laboratory, detailed in Figure 32 and elaborated in Table 8, demonstrates the ANN's strong predictive capability. Myristic acid and palmitic acid show close agreement between the ANN-predicted values (7.46% and 1.28%, respectively) and the experimental values (6.29% and 0.96%, respectively), highlighting the ANN's accuracy in predicting these specific fatty acids. While some discrepancies are noted for linolenic and linoleic acids, the overall alignment of total FAME values (8.78% ANN vs. 7.431% experimental) indicates the ANN effectively captures trends in fatty acid composition. This favorable outcome suggests that with further refinement, the ANN holds promise for enhancing predictive accuracy across all components of fatty acid composition analysis.

DISCUSSION

This research demonstrates the considerable potential of augmenting biodiesel production from microalgae via the

integration of Artificial Neural Network (ANN) modelling and experimental optimization. The strong correlation between the optimal acid concentration forecasted by the ANN model (0.5 M) and the experimental results, which similarly identified 0.5 M H_2SO_4 as the most effective concentration for biodiesel yield, highlights the dependability and strength of the ANN model in predicting essential parameters for biodiesel production. The integration of experimental methods with ANN modeling facilitates a comprehensive understanding of key factors such as acid concentration, temperature, stirring speed, and reaction time, which collectively contribute to the optimization of biodiesel yield. This research discovered optimal settings of 0.5 M H_2SO_4, 40°C, 400 RPM stirring speed, and a 2-hour reaction period, yielding 97.46% biodiesel. The experimental conditions were verified using High- Performance Liquid Chromatography (HPLC), confirming that the optimized settings maximized overall FAME (fatty acid methyl ester) content and successfully promoted the breakdown of essential fatty acids. This dual approach, combining experimental optimization with ANN modeling, ensures a reliable and precise method for enhancing biodiesel production processes. While the ANN model demonstrated high accuracy in predicting critical parameters such as reaction time and acid concentration (R2 = 0.94), minor discrepancies in the predicted values for temperature and stirring speed suggest areas for further refinement. These deviations highlight the complexity of the optimization process and indicate that future iterations of the ANN model could benefit from incorporating a broader range of experimental conditions, particularly regarding fine-tuning temperature and stirring speed predictions for more precise outcomes. While H_2SO_4 demonstrated efficacy as a catalyst for transesterification, its elevated cost and environmental repercussions pose significant questions about the long-term sustainability of large- scale biodiesel synthesis using this catalyst. This indicates a need for the investigation of alternative catalysts that have both economic and ecological advantages. Enzymatic hydrolysis, for example, presents a promising alternative with its sustainable profile, although its higher cost remains a limitation. Future research should focus on identifying and testing more cost-effective and sustainable catalytic options that can replace H_2SO_4 while maintaining or enhancing biodiesel yield. The economic benefits of ANN modeling are particularly noteworthy. By optimizing the experimental conditions, the model reduces dependence on laborious trial-and-error testing, resulting in considerable savings in time and money This predictive capability is especially valuable when scaling up biodiesel production, as optimizing key process parameters can lead to substantial cost reductions and improved yield. Consequently, ANN modelling functions as both an effective research instrument and a significant asset for industrial applications, enhancing the efficient scaling of biodiesel production and promoting more economical and sustainable energy solutions.

CONCLUSION

This study underscores the utility of combining experimental methods with ANN modeling to optimize biodiesel production processes. The alignment between experimental findings and ANN predictions for most parameters validates the effectiveness of the ANN model, while discrepancies in temperature and stirring speed warrant further investigation. The integration of HPLC analysis further strengthens the validation process, ensuring comprehensive and accurate optimization.

The optimal conditions identified for maximum biodiesel yield are a reaction time of 2 hours, a temperature of 40°C, a methanol-to-oil ratio of 12:1, a stirring speed of 400 RPM, and a sulfuric acid concentration of 0.5 M. HPLC analysis confirmed that these conditions not only maximize total FAME content but also effectively hydrolyze key fatty acids, providing a nuanced understanding of the hydrolysis process. Using data from the ANN model, the yield attained in the lab (98.1249%) was near to the anticipated yield (99.5315%) and higher than the experimental yield (97.461%), indicating the high accuracy and dependability of the ANN model's predictions. Future research should focus on the ANN model to further enhance the efficiency and yield of biodiesel production processes. These findings pave the way for more sustainable and cost-effective biodiesel production methods, contributing to the advancement of sustainable energy solutions.

ACKNOWLEDGEMENT

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DECLARATION OF COMPETING INTEREST

None.

REFERENCES

Abdullah, B., Syed Muhammad, S. A. F. ad, Shokravi, Z., Ismail, S., Kassim, K. A., Mahmood, A. N. & Aziz, M. M. A. 2019. Fourth generation biofuel: A review on risks and mitigation strategies. *Renewable and Sustainable Energy Reviews* 107: 37–50). https://doi.org/10.1016/j.rser.2019.02.018

Azizi, K., Keshavarz Moraveji, M. & Abedini Najafabadi, H. 2018. A review on bio-fuel production from microalgal biomass by using pyrolysis method. *Renewable and Sustainable Energy Reviews* 82: 3046–3059. https://doi.org/10.1016/j.rser.2017.10.033

Bautista, L. F., Vicente, G., Mendoza, Á., González, S., & Morales, V. 2015. Enzymatic production of biodiesel from nannochloropsis gaditana microalgae using immobilized lipases in mesoporous materials. *Energy and Fuels* 29(8): 4981–4989. https://doi.org/10.1021/ef502838h

Cao, H., Zhang, Z., Wu, X., & Miao, X. 2013. Direct biodiesel production from wet microalgae biomass of chlorella pyrenoidosa through in situ transesterification. *BioMed Research International* 2013. https://doi.org/10.1155/2013/930686

Cheah, W. Y., Er, A. C., Aiyub, K., Mohd Yasin, N. H., Ngan, S. L., Chew, K. W., Khoo, K. S., Ling, T. C., Juan, J. C., Ma, Z., & Show, P. L. 2023. Current status and perspectives of algae-based bioplastics: A reviewed potential for sustainability. *Algal Research* 71. https://doi.org/10.1016/j.algal.2023.103078

Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology Advances* 25(3): 294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chisti, Y. 2008. Biodiesel from microalgae beats bioethanol. *Trends in Biotechnology* 26(3): 126–131. https://doi.org/10.1016/j.tibtech.2007.12.002

Chuah, L. F., Klemeš, J. J., Yusup, S., Bokhari, A., & Akbar, M. M. 2017. A review of cleaner intensification technologies in biodiesel production. *Journal of Cleaner Production* 146: 181–193. https://doi.org/10.1016/j.jclepro.2016.05.017

Ebrahimpour, A., Rahman, R. N. Z. R. A., Ean Ch'ng, D. H., Basri, M., & Salleh, A. B. 2008. A modeling study by response surface methodology and artificial neural network on culture parameters optimization for thermostable lipase production from a newly isolated thermophilic Geobacillus sp. strain ARM. *BMC Biotechnology* 8. https://doi.org/10.1186/1472-6750-8-96

Fatiha, B., Sameh, B., Youcef, S., Zeineddine, D., & Nacer, R. 2013. Comparison of artificial neural network (ANN) and response surface methodology (RSM) in optimization of the immobilization conditions for lipase from Candida rugosa on amberjet® 4200-Cl. *Preparative Biochemistry and Biotechnology* 43(1): 33–47. https://doi.org/10.1080/10826068.2012.693899

Gulay Bayramoglu1, Aydin Akbulut, V. Cengiz Ozalp, and M. Yakup Arica. n.d. Immobilized lipase on micro-porous biosilica for enzymatic transesterification of algal oil. doi:10.1016/j.cherd.2014.12.011

Hidekl Fukuda, Akihiko Kond, & Hide Noda. 2001. Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bio Engineef*.

Kim, J., Yoo, G., Lee, H., Lim, J., Kim, K., Kim, C. W., Park, M. S., & Yang, J. W. 2013. Methods of downstream processing for the production of biodiesel from microalgae. *Biotechnology Advances* 31(6): 862–876. https://doi.org/10.1016/j.biotechadv.2013.04.006

Kusumo, F., Silitonga, A. S., Masjuki, H. H., Ong, H. C., Siswantoro, J., & Mahlia, T. M. I. 2017. Optimization of transesterification process for Ceiba pentandra oil: A comparative study between kernel-based extreme learning machine and artificial neural networks. *Energy* 134: 24–34. https://doi.org/10.1016/j.energy.2017.05.196

Liu, J., Zhou, F., Abed, A. M., Le, B. N., Dai, L., Elhosiny Ali, H., Khadimallah, M. A., & Zhang, G. 2023. Macroalgae as a potential source of biomass for generation of biofuel: Artificial intelligence, challenges, and future insights towards a sustainable environment. *Fuel* 336. https://doi.org/10.1016/j.fuel.2022.126826

Ma, F., & Hanna, M. A. (n.d.-b). Biodiesel production: Areview 1.

Megawati, Bahlawan, Z. A. S., Damayanti, A., Putri, R. D. A., Triwibowo, B., & Prasetiawan, H. 2022. Comparative study on the various hydrolysis and fermentation methods of chlorella vulgaris biomass for the production of bioethanol. *International Journal of Renewable Energy Development* 11(2): 515–522. https://doi.org/10.14710/IJRED.2022.41696

Mofijur, M., Atabani, A. E., Masjuki, H. H., Kalam, M. A., & Masum, B. M. 2013. A study on the effects of promising edible and non-edible biodiesel feedstocks on engine performance and emissions production: A comparative evaluation. In *Renewable and Sustainable Energy Reviews* 23: 391–404. https://doi.org/10.1016/j.rser.2013.03.009

Muhammad, G., Potchamyou Ngatcha, A. D., Lv, Y., Xiong, W., El-Badry, Y. A., Asmatulu, E., Xu, J., & Alam, M. A. 2022. Enhanced biodiesel production from wet microalgae biomass optimized via response surface methodology and artificial neural network. *Renewable Energy* 184: 753–764. https://doi.org/10.1016/j.renene.2021.11.091

Ong, M. Y., Nomanbhay, S., Kusumo, F., Raja Shahruzzaman, R. M. H., & Shamsuddin, A. H. 2021. Modeling and optimization of microwave-based bio-jet fuel from coconut oil: Investigation of response surface methodology (RSM) and artificial neural network methodology (ANN). *Energies* 14(2). https://doi.org/10.3390/en14020295

Rahman, M. A., Aziz, M. A., Al-khulaidi, R. A., Sakib, N., & Islam, M. 2017. Biodiesel production from microalgae Spirulina maxima by two step process: Optimization of process variable. *Journal of Radiation Research and Applied Sciences* 10(2): 140–147. https://doi.org/10.1016/j.jrras.2017.02.004

Rojo, E. M., Filipigh, A. A., & Bolado, S. 2023. Assisted-enzymatic hydrolysis vs chemical hydrolysis for fractional valorization of microalgae biomass. *Process Safety and Environmental Protection* 174: 276–285. https://doi.org/10.1016/j.psep.2023.03.067

Sathya, A. B., Thirunavukkarasu, A., Nithya, R., Nandan, A., Sakthishobana, K., Kola, A. K., Sivashankar, R., Tuan, H. A., & Deepanraj, B. 2023. Microalgal biofuel production: Potential challenges and prospective research. *Fuel* 332. https://doi.org/10.1016/j.fuel.2022.126199

Svozil, D., Kvasnieka, V., & Pospichal, J. 1997. Chemometrics and intelligent laboratory systems Introduction to multi-layer feed-forward neuralN networks. *Chemometrics and Intelligent Laboratory Systems* 39.

Timira, V., Meki, K., Li, Z., Lin, H., Xu, M., & Pramod, S. N. 2022. A comprehensive review on the application of novel disruption techniques for proteins release from microalgae. *Critical Reviews in Food Science and Nutrition* 62(16): 4309–4325. https://doi.org/10.1080/10408398.2021.1873734