



Biodiesel Production from *Chlorella* sp.

Danang Jaya^{1*}, Heni Anggorowati², Putri Restu Dewati³, Rifka Azzahra Artha Kinara⁴, and Niken Widiawati⁵

¹ Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta, 5598, Indonesia

² Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta, 5598, Indonesia

³ Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta, 5598, Indonesia

⁴ Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta, 5598, Indonesia

⁵ Faculty of Industrial Technology, Universitas Pembangunan Nasional "Veteran" Yogyakarta, 5598, Indonesia

* Correspondence: danangjay@upnyk.ac.id

Citation:

Jaya, D.; Anggorowati, H., Dewati, P.R.; Kinara, R.A.A.; Widiawati, N. Biodiesel production from *Chlorella* sp. *ASEAN J. Sci. Tech. Report.* **2025**, *28*(3), e256650. <https://doi.org/10.55164/ajstr.v28i3.256650>.

Article history:

Received: November 10, 2024

Revised: May 2, 2025

Accepted: May 12, 2025

Available online: May 31, 2025

Publisher's Note:

This article has been published and distributed under the terms of Thaksin University.

Abstract: This research utilizes the microalgae *Chlorella* sp. as a raw material for biodiesel production through the in-situ microwave-assisted transesterification method with the addition of a co-solvent. This study examines the effects of microwave power, reaction time, H₂SO₄ catalyst concentration on biodiesel yield. The study results show that using the in-situ microwave-assisted transesterification method with hexane as a co-solvent can increase the efficiency of biodiesel production from the microalgae *Chlorella* sp. The optimal conditions were a transesterification time of 7 minutes and a catalyst concentration of 0.3 M, which produced the highest biodiesel yield of 77.32%.

Keywords: Renewable energy; microalgae; biofuel; microwave; in situ transesterification

1. Introduction

Indonesia has several alternative energy potentials, including biogas, bioethanol, and biodiesel. Biodiesel has characteristics similar to those of fossil-based diesel engine fuels. It is environmentally friendly because it produces lower combustion emissions compared to petroleum-based diesel fuels and can decompose naturally. It is a renewable energy source [1, 2]. Biodiesel can be produced from various sources, such as vegetable oil, animal fat, and algae [3]. Microalgae, in particular, present a promising raw material for biodiesel production [4-5]. Among these, *Chlorella* sp. is known for its high lipid content, making it an ideal candidate for biodiesel feedstock. The oil content in *Chlorella* sp. is about 21.3%, with the major fatty acids being palmitic acid and oleic acid [6]. A practical method is needed to break down the rigid cell walls of the microalgae, allowing for more efficient oil extraction.

In situ transesterification is a cost-effective and straightforward method for biodiesel production [7-9]. This process, also known as direct transesterification [10], allows extraction and transesterification to coincide, with alcohol serving as both the solvent and the reactant [11]. Combining these steps, in situ transesterification eliminates the need for separate oil extraction and purification processes, making it highly efficient [7]. Further research is needed to explore the potential of *Chlorella* sp. microalgae for biodiesel production using in situ transesterification with an H₂SO₄ catalyst.

2. Materials and Methods

2.1 Materials

The microalgae (*Chlorella sp.*) used in this study were commercially sourced from PT. Spiralife Biotechnology Indonesia in dried powder form with a moisture content of approximately 5%. No further drying or particle size modification was conducted, and the biomass was used as received. Methanol (CH₃OH), n-hexane, ethanol, KOH, and a H₂SO₄ catalyst were obtained from Merck.

2.2 Methods

Ten grams of microalgae were placed in a 250 mL three-neck round-bottom flask, adding 150 ml of a hexane and methanol solvent mixture in a 3:2 (v/v) ratio. A catalyst was added according to the predetermined concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 N). The three-neck flask was then placed in the microwave. The flask was positioned in the center of the microwave cavity and stirred with a magnetic stirrer. Microwave-assisted in-situ transesterification was conducted at five power settings: 136 W, 264 W, 440 W, 616 W, and 800 W. Each run was performed for 3, 5, 7, 9, 11, or 13 minutes in continuous mode. No intervals or cooling periods were applied during the operation. After each run, the flask was cooled at room temperature (± 27 °C), and the temperature was measured using an infrared thermometer. The final mixture was then separated and analyzed.

3. Results and Discussion

3.1 Effect of Extraction Power Variation on Microalgae Oil Yield

The yield of microalgal oil influences the biodiesel yield produced. In the in situ process, the extraction and transesterification processes are carried out directly, meaning that it is not possible to analyze the yield of extracted microalgal oil using the in situ method. However, this study conducted a separate process to analyze the amount of oil that can be extracted from microalgae by varying the microwave power. This separate process was used to determine how much oil can be extracted and react to form biodiesel.

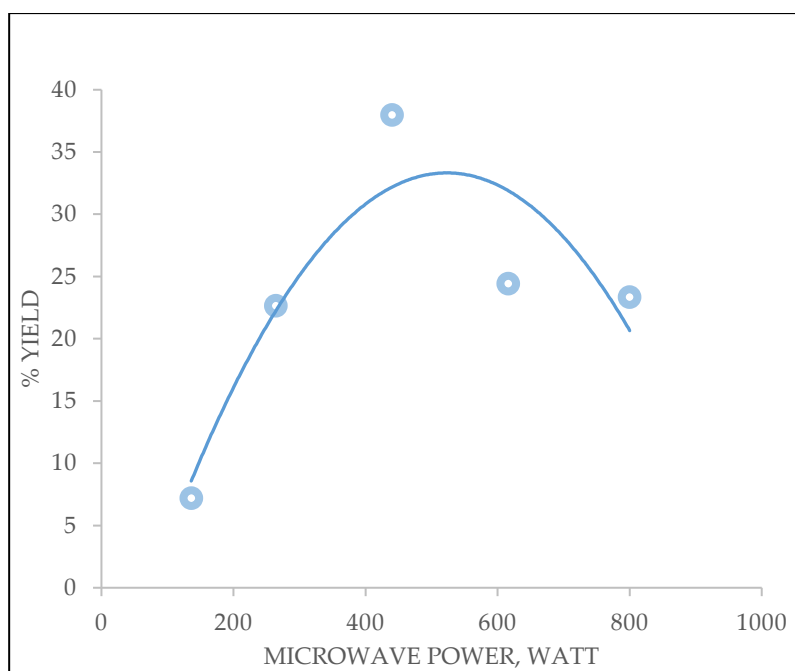


Figure 1. Relationship Between Microwave Power (136 watts, 264 watts, 440 watts, 616 watts, and 800 watts) and Microalgal Oil Yield Percentage.

Figure 1 illustrates the results of the extraction process at different microwave power levels: 136 watts, 264 watts, 440 watts, 616 watts, and 800 watts. Identifying the optimal microwave power for oil extraction from

Chlorella sp. maximizes oil yield. The findings indicate that the optimal power level, which produces the highest oil yield, is 440 watts. The data shows that 800-watt power provides a higher temperature than other powers.

Power is related to temperature [12], according to the formula:

$$P \cdot t = m \cdot c \cdot \Delta T \quad (1)$$

with: P = Microwave Power (Watt)

t = Time (s)

m = Mass of microalgae (kg)

c = specific heat ($\text{J kg}^{-1} \text{ } ^\circ\text{C}^{-1}$)

ΔT = temperature difference ($^\circ\text{C}$)

As shown in Equation 1, the relationship between power and temperature is directly proportional. This indicates that an increase in power leads to a more substantial change in temperature. High temperatures can help break down cell walls, facilitating contact between methanol and oil and producing higher yields. However, as indicated in Equation 1, a continuous increase in power will lead to even higher temperatures. Excessive heat at elevated temperatures may damage the biomass [13-15], causing it to dry out and potentially burn [16].

3.2 The Effect of Reaction Time on Biodiesel Yield

Figure 2 presents the results of a study examining the effect of transesterification time on biodiesel yield at a microwave power setting of 440 watts.

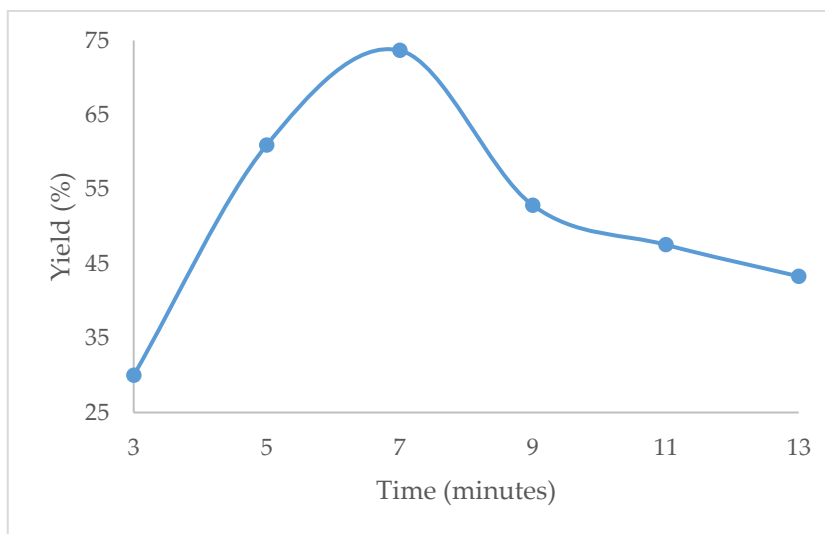


Figure 2. Effect of Extraction Time Variation (3, 5, and 7 minutes) on Biodiesel Yield

Figure 2 illustrates the results of the transesterification process conducted at a power setting of 440 watts. Biodiesel yield increased at 3, 5, and 7 minutes, with yields of 30%, 60.94%, and 73.66%, respectively. By the 7th minute, the biodiesel yield reached its optimum or equilibrium point, indicating that further reaction time would not enhance the yield. However, at the 9th minute, the yield decreased to 52.85%. Prolonged extraction times can result in excessive heating, significant solvent loss, the formation of by-products, and energy loss in the process. Compared to previous reports, the biodiesel yield of 73.66% obtained in this study after 7 minutes at 440 W is notably high. For instance, [17] reported a biodiesel yield of 68% from microalgae using microwave-assisted transesterification under similar conditions. Meanwhile, [18] achieved only 60% yield when using hexane as a co-solvent with waste cooking oil as feedstock. These differences may be attributed to the optimized microwave power applied in our study, the relatively short reaction time, and the synergistic effect of hexane as a co-solvent, which enhances methanol-lipid interaction. Furthermore, the direct use of *Chlorella sp.* with high lipid content and the simultaneous extraction-reaction strategy significantly

improve over conventional multistep methods. Integrating co-solvent and controlled microwave power likely contributed to better mass transfer and thermal efficiency, resulting in higher biodiesel yield within a shorter time frame.

The observed decline in yield at 9 minutes may be attributed to thermal degradation of fatty acid methyl esters (FAME) due to prolonged exposure to high temperatures. The excessive duration may also lead to methanol evaporation, decreasing reactant availability, or promoting side reactions such as soap formation and reverse esterification. These thermal and chemical instabilities likely reduced conversion efficiency beyond the optimal reaction time. Specifically, the decline in yield at the 9th minute may be attributed to thermal degradation of fatty acid methyl esters (FAME) due to prolonged exposure to microwave energy. Elevated temperatures can lead to ester breakdown, methanol evaporation, and reduced reactant availability. In addition, the extended heating period in the presence of an acid catalyst (H_2SO_4) may promote reverse reactions or unwanted side reactions such as saponification. These phenomena collectively reduce the efficiency of the transesterification process and result in a sharp decline in biodiesel yield after the optimal reaction time.

3.3 The Effect of H_2SO_4 Catalyst Concentration on Biodiesel Yield

This in situ transesterification process utilized microalgae powder, with methanol as solvent, hexane as co-solvent, and employed an acidic homogeneous catalyst, specifically H_2SO_4 . Methanol is the reactant due to its polar nature and short carbon chain. The homogeneous acid catalyst H_2SO_4 is more effective than base catalysts because it can produce a higher yield by converting free fatty acids (FFA) into fatty acid methyl esters (FAME). During the in situ transesterification process, the addition of a co-solvent, such as hexane, results in the formation of a single-phase solution. Although hexane and methanol do not typically mix, a sufficient volume of methanol creates a homogenous solution. As a co-solvent, hexane enhances the solubility of oil in methanol [17][18]. The addition of hexane reduces the interfacial surface tension between oil and methanol, forming a single-phase liquid. This improved mixing facilitates better contact between the reactants, thereby increasing the reaction rate. A single-phase solution is a key factor in the in situ transesterification process, as it significantly enhances the yield of crude biodiesel. Initial extraction was conducted by varying the extraction time to determine the optimum duration. The extraction process involved 10 grams of powdered microalgae and time variations of 3, 5, 7, 9, 11, and 13 minutes, carried out at a microwave power setting of 440 watts, resulting in the highest yield at 7 minutes. Following extraction, transesterification was performed using a fixed biomass-to-methanol ratio of 1:15 (wt/vol) while varying the catalyst concentration. The study commenced by weighing 10 grams of microalgae powder and mixing it with methanol and hexane as co-solvents, followed by the addition of H_2SO_4 catalyst in varying concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 M.

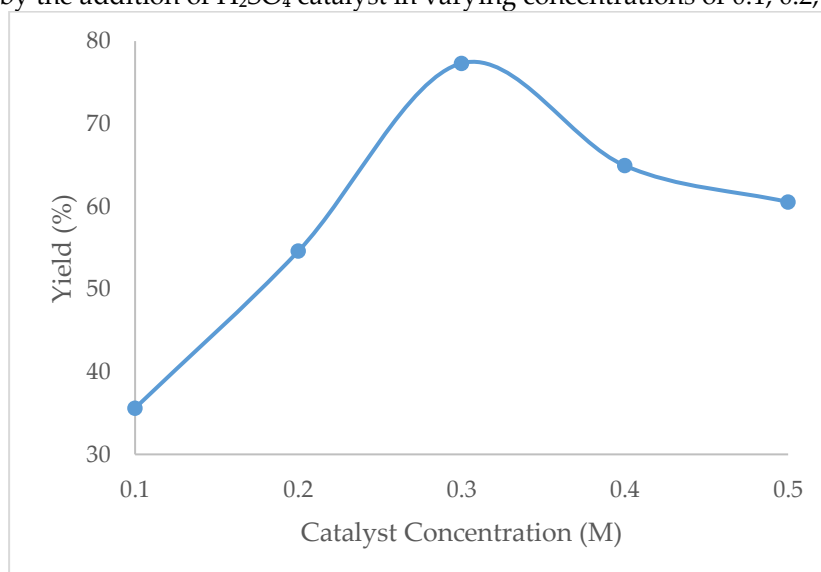


Figure 3. The Effect of H_2SO_4 Catalyst Concentration (0.1, 0.2, 0.3, 0.4, and 0.5 M) on Biodiesel Yield

Figure 3 illustrates the effect of varying H_2SO_4 catalyst concentrations on biodiesel yield. The respective yields at catalyst concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 M were 35.6%, 54.59%, 77.32%, 64.93%, and 60.53%. The results indicate that increasing the concentration of the H_2SO_4 catalyst from 0.1 M to 0.3 M leads to a substantial increase in biodiesel yield, peaking at 77.32%. This trend aligns with findings from [19], who reported that higher concentrations of H_2SO_4 catalyst reduce the percentage of free fatty acids (%FFA), thereby enhancing biodiesel production. However, as the catalyst concentration is increased beyond 0.3 M, a decline in yield is observed. This reduction may be attributed to the adverse effects of high acid catalyst concentrations, which can lead to alcohol dehydration. Such dehydration can inhibit the transesterification process, reducing overall biodiesel yield.

3.4 GC-MS Analysis Results

From the analysis, the following data was obtained:

Table 1. Results of GC-MS Analysis of Biodiesel

Compounds Analyzed	Amount (%)
9-Octadecanoic acid	19.24
Benzene, 1-ethyl-2-methyl	3.83
Decane	1.67
Hexadecanoic acid, methyl ester	10.06
Hexadecanoic acid	1.47
13-Hexyloxacyclotridec-10-en-2-one	5.65
9-Octadecenoic acid, methyl ester	46.34
Octadecenoic acid, methyl ester	11.73

Table 1 shows that the biodiesel content in the reaction product is 68.14%, with a remaining free fatty acid content of 20.72%. The remainder consists of other compounds, such as 13-Hexyloxacyclotridec-10-en-2-one, an antioxidant compound at 5.65%. The GC-MS analysis revealed that the biodiesel obtained contains 68.14% FAMEs, which does not meet the minimum standard of 96.5% set by SNI 7182:2015. This indicates that additional purification steps are necessary to enhance fuel quality before it can be applied in diesel engines. Future studies should focus on improving the transesterification efficiency and integrating purification techniques to meet the standard biodiesel quality.

4. Conclusions

The study results showed that using the in-situ microwave-assisted transesterification method with hexane co-solvent can increase the efficiency of biodiesel production from the microalgae *Chlorella sp.* The optimal conditions were found at a transesterification time of 7 minutes and a catalyst concentration of 0.3 M, which produced the highest biodiesel yield of 77.32%. Future research should explore the other variables of optimization of process parameters and evaluate the physicochemical properties of the biodiesel, including cetane number, viscosity, density, thermal, and oxidative stability. Additionally, studies focusing on the scale-up potential of this method, as well as a thorough techno-economic analysis, are essential for evaluating its feasibility in industrial applications. These aspects, which were beyond the scope of the present work, are recommended for future investigation.

5. Acknowledgements

The authors would like to thank to Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Universitas Pembangunan Nasional Veteran Yogyakarta for providing financial support for the implementation of this research

Author Contributions: Conceptualization, D.J., and H.A.; methodology, D.J., H.A. and P.R.D.; validation, D.J., H.A. and P.R.D.; formal analysis, D.J., H.A., P.R.D. R.A.A.K. and N.W.; investigation, H.A., P.R.D., R.A.A.K. and N.W.; resources, H.A. and P.R.D.; data curation, D.J., R.A.A.K. and N.W.; writing-original manuscript preparation, H.A. and P.R.D.; visualization, H.A. and P.R.D.; supervision, D.J. and H.A.; funding acquisition, D.J., H.A. and P.R.D. All authors have read and agreed the manuscript.

Funding: This research was funded by a grant from Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Universitas Pembangunan Nasional Veteran Yogyakarta, under a basic research scheme with contract number 121/UN62.21/DT.07.00/2024.

Conflicts of Interest: The authors declare no conflict of interest.

References

- [1] Edeh, I. Biodiesel Production as a Renewable Resource for the Potential Displacement of the Petroleum Diesel. In *Biorefinery Concepts, Energy and Products*; Beschkov, V., Ed.; IntechOpen: London, 2020. <https://doi.org/10.5772/intechopen.93013>
- [2] Rezanía, S.; Taib, S. M.; Din, M. F. M.; Dahalan, F. A.; Kamyab, H.; Ariffin, H.; Montoya, L. H.; Mohammadi, A. A. Review on Transesterification of Non-Edible Sources for Biodiesel Production with a Focus on Economic Aspects, Fuel Properties and By-Product Applications. *Energy Convers. Manag.* **2019**, *201*, 112155. <https://doi.org/10.1016/j.enconman.2019.112155>.
- [3] Hassan, M. H.; Kalam, M. A. An Overview of Biofuel as a Renewable Energy Source: Development and Challenges. *Procedia Eng.* **2013**, *56*, 39-53. <https://doi.org/10.1016/j.proeng.2013.03.087>.
- [4] Zhang, S.; Zhang, L.; Xu, G.; Li, F.; Li, X. A Review on Biodiesel Production from Microalgae: Influencing Parameters and Recent Advanced Technologies. *Front. Microbiol.* **2022**, *13*, 970028. <https://doi.org/10.3389/fmicb.2022.970028>.
- [5] Pandey, S.; Narayanan, I.; Selvaraj, R.; Varadavenkatesan, T.; Vinayagam, R. Biodiesel Production from Microalgae: A Comprehensive Review on Influential Factors, Transesterification Processes, and Challenges. *Fuel* **2024**, *367*, 131547. <https://doi.org/10.1016/j.fuel.2024.131547>.
- [6] Mathimani, T.; Sekar, M.; Shanmugam, S.; Sabir, J. S. M.; Chi, N. T. L.; Pugazhendhi, A. Relative Abundance of Lipid Types among *Chlorella* sp. and *Scenedesmus* sp. and Ameliorating Homogeneous Acid Catalytic Conditions Using Central Composite Design (CCD) for Maximizing Fatty Acid Methyl Ester Yield. *Sci. Total Environ.* **2021**, *771*, 144700. <https://doi.org/10.1016/j.scitotenv.2020.144700>.
- [7] Go, A. W.; Sutanto, S.; Ong, L. K.; Tran-Nguyen, P. L.; Ismadji, S.; Ju, Y. H. Developments in In-Situ (Trans) Esterification for Biodiesel Production: A Critical Review. *Renew. Sustain. Energy Rev.* **2016**, *60*, 284-305. <https://doi.org/10.1016/j.rser.2016.01.070>.
- [8] Kasim, F. H.; Harvey, A. P.; Zakaria, R. Biodiesel Production by In Situ Transesterification. *Biofuels* **2010**, *1*(2), 355-365. <https://doi.org/10.4155/bfs.10.6>.
- [9] Rodrigues, M. C.; Cunha, S.; Teixeira, L. S. G. In Situ Transesterification from Soybean Seed Using Mechanochemical Methods Toward Producing Biodiesel. *ACS Omega* **2023**, *8*(50), 47791-47797. <https://doi.org/10.1021/acsomega.3c06269>.
- [10] Makareviciene, V.; Sendzikiene, E.; Gumbyte, M. Application of Simultaneous Oil Extraction and Transesterification in Biodiesel Fuel Synthesis: A Review. *Energies* **2020**, *13*(9), 2204. <https://doi.org/10.3390/en13092204>.
- [11] Tuntiwiwattanapun, N.; Monono, E.; Wiesenborn, D.; Tongcumpou, C. In-Situ Transesterification Process for Biodiesel Production Using Spent Coffee Grounds from the Instant Coffee Industry. *Ind. Crops Prod.* **2017**, *102*, 23-31. <https://doi.org/10.1016/j.indcrop.2017.03.019>.
- [12] Amarulloh, A.; Haikal, H.; Atmoko, N. T.; Utomo, B. R.; Setiadhi, D.; Marchant, D.; Zhu, X.; Riyadi, T. W. B. Effect of Power and Diameter on Temperature and Frequency in Induction Heating Process of AISI 4140 Steel. *Mech. Eng. Soc. Ind.* **2022**, *2*(1), 26-34. <https://doi.org/10.31603/mesi.6782>.
- [13] Ali, M.; Watson, I. A. Microwave Thermolysis and Lipid Recovery from Dried Microalgae Powder for Biodiesel Production. *Energy Technol.* **2016**, *4*(2), 319-330. <https://doi.org/10.1002/ente.201500242>.

-
- [14] Gil-Martín, E.; Forbes-Hernández, T.; Romero, A.; Cinciosi, D.; Giampieri, F.; Battino, M. Influence of the Extraction Method on the Recovery of Bioactive Phenolic Compounds from Food Industry By-Products. *Food Chem.* **2022**, 378, 131918. <https://doi.org/10.1016/j.foodchem.2021.131918>.
- [15] Chaves, J. O.; de Souza, M. C.; da Silva, L. C.; Lachos-Perez, D.; Torres-Mayanga, P. C.; da Fonseca Machado, A. P.; Forster-Carneiro, T.; Vázquez-Espinosa, M.; González-de-Peredo, A. V.; Barbero, G. F.; Rostagno, M. A. Extraction of Flavonoids from Natural Sources Using Modern Techniques. *Front. Chem.* **2020**, 8, 507887. <https://doi.org/10.3389/fchem.2020.507887>.
- [16] Bintari, Y. R.; Haryadi, W.; Rahardjo, T. J. Ekstraksi Lipida dengan Metode Microwave Assisted Extraction dari Mikroalga yang Potensial sebagai Biodiesel. *JU-ke* **2018**, 2(2), 180-189.
- [17] Qadariyah, L.; Mujaddid, F.; Bhuana, D. S.; Mahfud, M. Biodiesel Production from Microalgae with Trans-Esterification Method Using Microwave. *IOP Conf. Ser. Mater. Sci. Eng.* **2019**, 543(1), 012073. <https://doi.org/10.1088/1757-899X/543/1/012073>.
- [18] Mohadesi, M.; Moradi, G.; Ghanbari, M.; Moradi, M. J. Investigating the Effect of n-Hexane as Solvent on Waste Cooking Oil Conversion to Biodiesel Using CaO on a New Support as Catalyst. *Meas. J. Int. Meas. Confed.* **2019**, 135, 606-612. <https://doi.org/10.1016/j.measurement.2018.12.022>.
- [19] Ramadhas, A. S.; Jayaraj, S.; Muraleedharan, C. Biodiesel Production from High FFA Rubber Seed Oil. *Fuel* **2005**, 84(4), 335-340. <https://doi.org/10.1016/j.fuel.2004.09.016>.