



Performance of In-Situ Stirring Batch Reactor Transesterification of *Nannochloropsis sp* Microalgae into Biodiesel

Yustia Wulandari Mirzayanti^{1*}, Lenny Marlinda², Hery Irawan³, Muhammad Al Muttaqii⁴, Zuhdi Ma'sum⁵, Nyoman Puspa Asri⁶, Jia-Ming Chern⁷

¹Department of Chemical Engineering, Faculty of Technology Industrial, Institut Teknologi Adhi Tama Surabaya, Surabaya 60117, Indonesia

²Department of Industrial Chemistry, University of Jambi, Jambi 36361, Indonesia

³Department of Mechanical Engineering, Faculty of Technology Industrial, Institut Teknologi Adhi Tama Surabaya, Surabaya 60117, Indonesia

⁴Research Center for Chemistry, Badan Riset dan Inovasi Nasional, South Tangerang 15314, Indonesia

⁵Department of Chemical Engineering, University of Tribhuwana Tungga Dewi, Malang 65144, Indonesia

⁶Department of Food Technology, University of Ciputra, Surabaya, 60219, Indonesia

⁷Department of Chemical Engineering and Biotechnology, Tatung University, Taipei, 10491, Taiwan

Abstract *In-situ* stirring batch reactor transesterification is a widely used method for the conversion of various biomass into biodiesel, particularly *Nannochloropsis sp* microalgae. Therefore, this study aims to investigate the effect of CaO/Hydrotalcite catalyst loading ratio and stirring rate on *in situ* stirring batch reactor transesterification of *Nannochloropsis sp* microalgae to produce biodiesel. *In-situ* transesterification was carried out using stirring with a four-blade paddle (50 w), while CaO/Hydrotalcite catalyst preparation was performed using incipient wetness impregnation. *Nannochloropsis sp* microalgae were then converted into biodiesel with varying loading ratio of catalyst (1:1, 3:1, and 5:1 %wt/wt) and stirring speed of agitation (50, 100, 150, 200, and 250 rpm) for 4 hours (60°C). Subsequently, biodiesel obtained from the process was analyzed using gas chromatography (GC). The results showed that the surface area of CaO/Hydrotalcite was 45.756 m²/g. The conversion yields of *Nannochloropsis sp* microalgae to biodiesel with CaO/Hydrotalcite reached 38.36% with an agitation rate of 250 rpm. Based on the fatty acids analyzed using GC methods, the products predominantly contained olefine (61.49%) and gasoline (38.51%).

Keywords: Biodiesel; CaO/Hydrotalcite; *In-Situ* transesterification; *Nannochloropsis sp*.

1. Introduction

Renewable energy, such as biofuels, is a valuable alternative to fossil fuels due to its beneficial properties. In addition, biofuels are classified into generations based on the raw materials used during their synthesis, including edible and non-edible oils, biomass, lignocellulose, and residues (Lomeu *et al.*, 2023; Asri *et al.*, 2021; Budianto *et al.*, 2018). Among the raw materials, microalgae are biomass with the potential to serve as a feedstock, primarily due to their high lipid content, accelerated growth, and higher photosynthetic biomass yield compared to land plants (Larida and Bañaga, 2021; Shirazi *et al.*, 2020). In line with previous studies, microalgae primarily comprise starch, proteins, lipids,

*Corresponding author's email: yustiawulandari_che@itats.ac.id, Tel.: +62-31-5997244
doi: [10.14716/ijtech.v15i4.6678](https://doi.org/10.14716/ijtech.v15i4.6678)

triglycerides, and fatty acids, particularly for biofuels production. These algae also offer various potential benefits over lignocellulosic source crops due to the absence of competition for agricultural land with other food crops. However, a notable challenge lies in their rigid cell wall structure, which typically requires solvent for extraction compared to vegetable oils (Sardi *et al.*, 2022; El-Sheekh, 2021; Purkan *et al.*, 2021; Culaba *et al.*, 2020; Cercado, Ballesteros, and Capareda, 2018). In response to this challenge, several technologies have been developed for the conversion of microalgae biomass and are classified into different types, including biochemical conversion, thermochemical conversion, chemical reaction, and direct combustion.

According to previous studies, biodiesel is predominantly produced using transesterification method, which comprises mixing lipid (triglyceride) and alcohol, with catalysts being used to facilitate the synthesis of ester and glycerol. High free fatty acids (FFA) cannot be directly transesterified using an alkaline catalyst, which gives low yield and low quality of biodiesel (Wahyono *et al.*, 2022). The best biodiesel production method typically comprises the use of lipid with carbon chain length ranging from saturated C₁₄ to C₂₂, reduced unsaturated free fatty acids groups, and lower saturation states. This is primarily because the use of high unsaturation levels can negatively affect the crucial aspect of the product obtained (Koech, Kumar, and Siagi, 2020). Consequently, several reports have explored the use of *Nannochloropsis sp.*, which has high oil content and can serve as a suitable green and low-impact environmental alternative fuel. This organism has also been reported to possess various advantages, such as biodegradability, nontoxicity, renewability, and safety.

Several studies have explored the conventional conversion of *Nannochloropsis sp.* microalgae to biodiesel using extraction with solvents, followed by transesterification process (Ghedini *et al.*, 2022; Makareviciene and Sendzikiene, 2022; Purkan *et al.*, 2021; Wood, 2021; Kazemifard *et al.*, 2019; Salam *et al.*, 2016). For example, a previous investigation used NiO nanocatalyst for in-situ transesterification synthesis of biodiesel from *Nannochloropsis oculata*, consisting of 2 stages, including in-situ extraction and transesterification (Purkan *et al.*, 2021; Salam *et al.*, 2016). However, this conventional method typically requires a high conversion cost. To overcome the limitation, various reports have proposed the use of in-situ transesterification, where extraction and esterification processes are combined to save time and enhance efficiency. In this method, the alcohol compound serves as both a solvent to extract lipids and a reactant for esterification reaction. In addition, co-solvents and catalysts can also be added to facilitate rate of transesterification reaction. Despite the potential, the effect of stirring rate parameters (Purkan *et al.*, 2021) on biodiesel yield has not been explored (Salam, Velasquez-Orta, and Harvey, 2016). An in-depth understanding of the impact of mixing on transesterification process has been reported to be an essential parameter in the scaling-up and design of reactors (Tabatabaei *et al.*, 2019). Industrial agitators and mixers for biodiesel production process often include anchor agitators, helix mixers, high-shear mixers, magnetic stirrers, mechanical stirrers, turbines agitators, micro-mixers, motionless or static mixers, two flat-blade paddle agitators, and ultrasonic mixers.

Heterogeneous catalyst are gaining more interest because its have advantages over homogeneous catalysts (Aisyah *et al.*, 2023; Rahma and Hidayat, 2023; Cercado, Ballesteros, and Capareda, 2018). Based on previous studies, calcium oxide (CaO) is a base heterogeneous catalyst with good potential for chemical reaction, particularly transesterification reaction (Zul *et al.*, 2021). Catalyst has also been shown to have high alkalinity, low solubility, and can easily be separated from the product. However, CaO has a weak catalytic activity, leading to reduced product yield. This is because the oxygen ions (O²⁻) on its surface can quickly form hydrogen bonds with methanol or glycerol,

necessitating the support of an active metal, such as hydrotalcite. According to previous studies, hydrotalcite is a hydroxycarbonate form of magnesium and aluminum with the molecular formula $Mg_6Al_2(OH)_{16}CO_3 \cdot 4H_2O$ and is also a derivative of $Mg(OH)_2$ (Mirzayanti, Alisa, and Sari, 2020a). The use of this compound as catalyst in transesterification reaction has the potential to increase biodiesel yield (Zhang *et al.*, 2019). Therefore, it is necessary to combine CaO with hydrotalcite to improve its catalytic performance.

The novelty in this current study lies in the use of a metal catalyst (CaO/Hydrotalcite) to convert *Nannochloropsis sp* microalgae into biodiesel using in-situ transesterification method as well as exploring the effectiveness of the agitation rate parameters on reactor batch in-situ transesterification. CaO is an alkali metal oxide with various advantages, while hydrotalcite is a heterogeneous catalyst that can accelerate transesterification reaction. The investigation primarily focuses on the performance of the combination of CaO metal and hydrotalcite as catalyst. Reactor's design was *in-situ* stirred batch reactor with a unique design comprising a four-blade paddle. Catalyst used was an alkali metal, which comprised the combination of CaO/Hydrotalcite heterogeneous catalyst and methanol as a solvent and a reactant in in-situ transesterification process, with n-hexane serving as a co-solvent. Therefore, this study aims to determine the effect of CaO/Hydrotalcite loading ratio and stirring rate in converting *Nannochloropsis sp* microalgae.

2. Methods

This section described the principal materials and the methodologies used in the experimental procedures.

2.1. Material, Equipment, and Apparatus

Nannochloropsis sp microalgae were obtained from the Brackish Water Aquaculture Fisheries at Jepara, Central Java, Indonesia. Meanwhile, calcium carbonate, methanol, n-hexane (Merck & Co, 99%), and hydrotalcite synthetic (Sigma Aldrich, 99%) were purchased from an Indonesian supplier. The major equipment was in-situ stirred batch reactor (material: stainless steel type 304), vertical cylindrical shape equipped with a stirrer. The top and bottom covers were flange-shaped, with a total tank volume of 20 liters and a stirrer of 50 w (four-blade paddle) (Figure 1a). Figure 1b illustrated a stirred tank batch reactor with dimensions of outside diameter (d_o) = 7.48 in; cylinder height (L_s) = 7.48 in; cylinder thickness (t_s) = 3/16 in; and leg support (l) = 20 in (Scale 1:1). The heating circulation had a maximum speed of 500 rpm, a maximum temperature of 250°C, and H of 20.5 in (scale = 1:1).

2.2. Synthesis and Characterization of CaO/Hydrotalcite

The incipient wetness impregnation method was used for the synthesis of the CaO/Hydrotalcite catalyst, which was placed in an oven for 12 hours at 100°C. The calcium carbonate ($CaCO_3$) was calcined into CaO for 3 hours at 900°C and then weighed with loading ratio of CaO to hydrotalcite (1:1, 3:1, and 5:1 wt/wt). Subsequently, CaO was dissolved in distilled water, and the solution was sprayed onto hydrotalcite slowly and evenly until it seeped into hydrotalcite pores at room temperature. The sample was dried at 100°C for 12 h and treated in the furnace at 900°C for 3 hours. The final product was CaO/Hydrotalcite catalyst powder, which was characterized using *X-ray diffraction* (XRD), *X-ray Fluorescence* (XRF), and *Brunaur Emmett and Teller* (BET) to investigate the surface area. The characterization techniques of XRD investigations were performed using instruments possessing Cu K-Alpha source, 40 kV and 30 mA generator settings, and no beam monochromator.

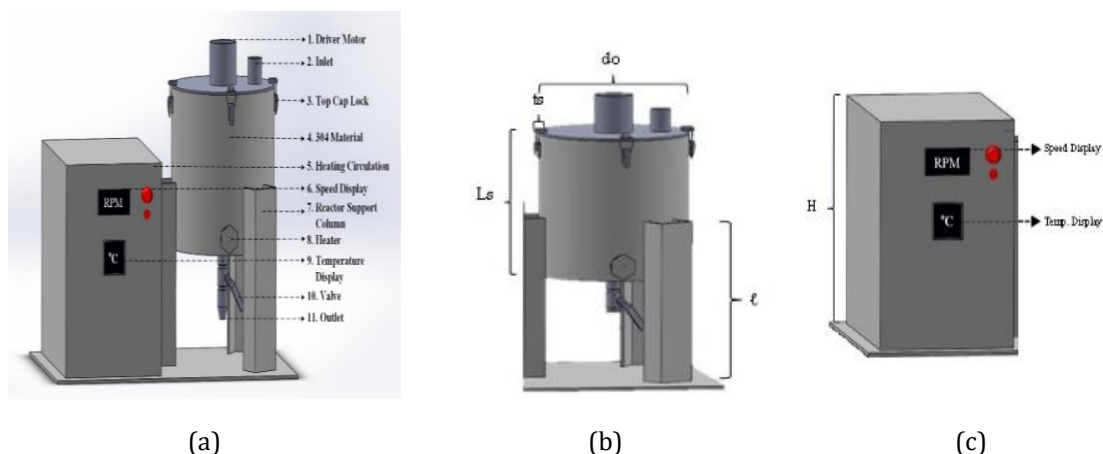


Figure 1 a) *In Situ* stirred batch reactor transesterification, b) stirred tank batch reactor, and c) heat circulation

2.3. *In-Situ* Transesterification Reaction

The experimental setup consisted of in-situ stirred batch reactor, as shown in Figure 1. The weighed amount of *Nannochloropsis sp* microalgae biomass was mixed with CaO/Hydrotalcite catalyst (10%wt of microalgae) and methanol. The mixture obtained was then blended for 4 hours at 60°C with varying agitation rates of 50, 100, 150, 200, and 250 rpm. After the reaction had occurred, it was cooled to separate biodiesel from glycerol and suspended solids. Subsequently, the filtered residue was washed using 30 mL of a mixture of methanol-n-hexane (1:1 v/v) to obtain fatty acids methyl ester (FAME). The filtrate was separated by adding n-hexane with a 1:1 (v/v) ratio, shaken, and allowed to stand until 2 layers formed for 30 minutes. The bottom layer was then removed from the separating, and n-hexane was added with a 1:1 (v/v) ratio. The solution was shaken and allowed to stand until 2 layers formed for 8 hours. The top layer was washed to remove any catalyst and glycerol in the mixture. The product obtained was distilled (70°C) to separate FAME and solvent. *Gas Chromatography-Mass Spectrometry* (GC-MS) was used to analyze biodiesel for detected fatty acids in higher yields in all variables. The peak area from GC-MS data could be read by the normalization method based on the peak area per percentage of identified components used to measure the GC-MS results. All hydrocarbon components were identified using Wiley275 and NIST02 mass spectral data libraries. Hydrocarbon components with a probability equal to or above 80% were considered in line with previous studies (Marlinda *et al.*, 2022; Mirzayanti *et al.*, 2020b). This current study optimized the process variables for the highest yield, namely the effectivity of the speed of stirring conversion to crude biodiesel and loading of CaO/Hydrotalcite catalyst by microalgae, density, acid number, and %FFA. The limitation of this report was that it solely focused on the effect of the CaO and hydrotalcite loading ratio and stirring speed on yield and FFA. The best yield obtained was subjected to GC-MS testing to determine the content of hydrocarbon compounds produced.

2.4. Analysis of Crude Biodiesel

Analysis and determination of the %yield of crude biodiesel was calculated with the formulations presented below (Equation 1)

$$\text{Yield crude bioiesel (\%)} = (M_B/M_A) \times 100 \quad (1)$$

where M_B is the mass of crude biodiesel (g); and M_A is microalgae (g) mass. Compound analysis biodiesel components (ASTM D4007) were analyzed using GC-MS.

3. Results and Discussion

3.1. Characteristics of *Nannochloropsis sp* Microalgae

Characteristics of lipid of liquid *Nannochloropsis sp* microalgae biomass are presented in Table 1. The results showed that the highest fatty acids content in *Nannochloropsis sp* microalgae was oxygenated compounds (51.80%), followed by carboxylic acid (46.61%). In addition, oxygenated compounds were hydrocarbons containing at least 1 oxygen atom as a part of their chemical structure, and are commonly referred to as fuels. The 4 general classes of hydrocarbons included alkanes, alkenes, alkynes, and arenes. Carboxylic acid, in organic chemistry, is an organic acid containing a carboxyl group (C(=O)OH) attached to an R-group. This compound was characterized by its carbon (C) atom being bonded to an oxygen (O) atom by a double bond as well as to a hydroxyl group (–OH) by a single bond. Based on Table 1, *Nannochloropsis sp* microalgae could be used as raw materials for biodiesel.

Table 1 Fatty acid content in *Nannochloropsis sp* microalgae

No	Component	%area
1	Alkenes/olefins	1.59
2	Oxygenated compounds	51.80
3	Carboxylic acid	46.61

The total lipid content of microalgae biomass largely depended on the specific cultivation technology. Microalgae culture condition, nutrients, and light intensity could be optimized to increase the oil content, leading to enhanced biodiesel production. In addition, the growth rate of this organism and maximum biomass production depended on their culture systems (light, temperature, pH, salinity, consumption of O₂, CO₂, nutrients, and toxic chemicals) (Medipally *et al.*, 2015). According to previous studies, nutrients for microalgae growth included nitrogen (N) and/or phosphorus (P) (Kazemifard *et al.*, 2019). *Nannochloropsis sp* microalgae could serve as biodiesel due to the abundance of its oleic acid content (73.40%) among the remainder of the MUFA composition (Kanagesan *et al.*, 2020). The specific growth rate of *Nannochloropsis sp* microalgae strains generally ranged from 0.11 to 0.21 per day, with total lipid content of 37-60% of dry weight (DW) (Ma *et al.*, 2016).

3.2. Catalyst Characterization

Figure 2 showed a series of XRD diffractions of CaO/Hydrotalcite. The initial incipient wetness impregnation at 900°C in 3 hours predominantly contained CaO and MgO with few amounts of Al₂O₃. In addition, the XRD pattern of CaO/Hydrotalcite catalyst had related curves in terms of uniformity. The peaks obtained were compared with the Joint Committee on Powder Diffraction Standards (JCPDS) file. From Figure 3, the principal components of CaO/Hydrotalcite catalyst included CaO, Ca₂Al, and MgO. Diffraction 2θ from JCPDS for using CaO was 32.2°, 37.3°, and 53.8°. Therefore, the pattern in Figure 2 showed the intensified and narrow peaks at 2θ = 17.88°, 28.47°, 33.92°, 42.88°, 47.07°, and 50.60°, as reported in a previous study (Mohamad *et al.*, 2018). Catalyst characterization also used XRF analysis to determine the chemical compositions. The elemental chemical compositions of catalyst in this study are presented in Table 2. According to the XRF, the CaO content of the various ratio of CaO with hydrotalcite (1:1, 3:1, and 5:1 %wt/wt) increased gradually. The highest amount of CaO was obtained at ratio of 5:1 %wt/wt (84.13 %m/m), as shown in Table 2. The results suggested that the decomposition of carbonates was achieved at 900°C for approximately 3 hr. In a previous study, the majority of variation ratio catalyst above 50% were CaO species (Amusan *et al.*, 2019). The total content of MgO

and Al_2O_3 was below 50% because hydrotalcite was decomposed into metal oxides when calcined at 900°C for 3 hours (Amusan *et al.*, 2019; Mohamad *et al.*, 2018).

The surface area of CaO/Hydrotalcite particles synthesized to catalyst (obtained by calcination at 900°C for 3 h) from BET and BJH was $45.756\text{ m}^2/\text{g}$. Furthermore, the surface area of the obtained catalyst showed good activity for converting *Nannochloropsis sp* oil into biodiesel. This result was consistent with a previous study, which distributed CaO or K_2CO_3 in various support materials (Manurung *et al.*, 2023; Zhang *et al.*, 2019). Navajas *et al.* (2018) also reported the best results for hydrotalcite activity as a heterogeneous methanolysis catalyst. These outcomes were attributed to the high basicity properties of the solid that was subjected to the rehydration process after calcination with a surface area of 12 and $66\text{ m}^2/\text{g}$. Apart from the strong basic sites, the presence of interlayer hydroxide anions at the accessible crystal edges enhanced catalyst's activity. However, hydrotalcite synthesized using a variety of processes had a surface area of $131\text{--}153\text{ m}^2/\text{g}$ at pH 10 and 150°C (Navajas *et al.*, 2018). This result was better than previous studies, which obtained an average value of $34.6\text{ m}^2/\text{g}$. The CaO/Hydrotalcite area was more significant compared to a previous study at $4.34\text{ m}^2/\text{g}$ (Win and Khine, 2017).

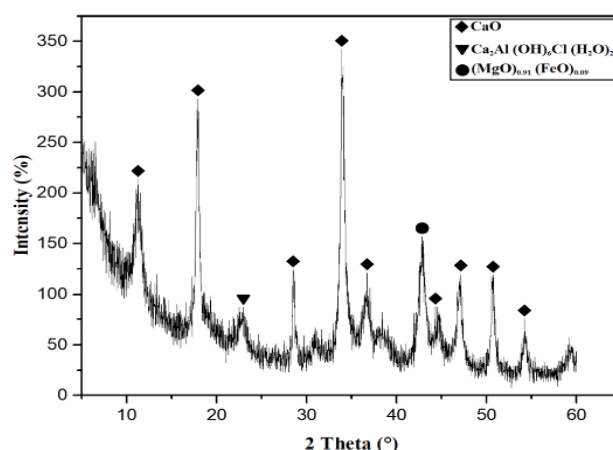


Figure 2 XRD diffractogram of CaO/Hydrotalcite calcined at 900°C for 3 h (5:1 %wt/wt)

Table 2 XRF analysis of CaO/Hydrotalcite at 900°C for 3 hours

No	Component	%Massa (m/m)		
		1:1	3:1	5:1
1	CaO	55.05	66.80	84.13
2	MgO	32.33	24.08	11.76
3	Al_2O_3	12.35	8.90	4.00
4	SiO_2	0.17	0.11	0.02
5	Na_2O	0.08	0.10	0.08
6	Fe_2O_3	0.01	0.01	0.00
7	SO_3	0.01	0.01	0.01

3.3. Catalytic Performance for Biodiesel Production

In-situ stirring batch reactor transesterification reaction was performed using CaO/Hydrotalcite, and the reaction conditions were investigated by varying the different parameters. The effect of stirring speed and CaO loading on transesterification was also studied in the reaction.

3.3.1. Effect of stirring rate and CaO loading on %yield crude biodiesel

Figure 3a presents stirring speed of the different CaO/Hydrotalcite ratio of CaO/Hydrotalcite catalyst. In addition, the highest yield of crude biodiesel at 250rpm was

obtained with a 1:1 wt/wt ratio of catalyst (38.36%). A high mixing must be used during *in situ* transesterification to obtain high FAME and yield (Salam, Velasquez-Orta, and Harvey, 2016). The results showed that the yield increased with an increase in mixing speed. The process showed that the mixing speed at 250 rpm was 38.36%. Figure 3 also showed the presence of an anomaly in the decrease of %yield crude biodiesel of CaO/Hydrotalcite catalyst (1:1 %wt/wt; 150 rpm and 3:1 %wt/wt; 100 and 200 rpm). Loading ratio of CaO/Hydrotalcite catalyst at 1:1 produced the highest yield. This was due to the best metal composition in the 1:1 %wt/wt composition. When catalyst loading ratio was increased (5:1 %wt/wt), the results showed a decrease in %yields, as reported in previous studies (Koech, Kumar, and Siagi, 2020). The outcome observed was caused by interaction with other molecules/compounds, leading to increased by-products. The high catalyst concentration led to a corrosive nature, which hindered transesterification reaction.

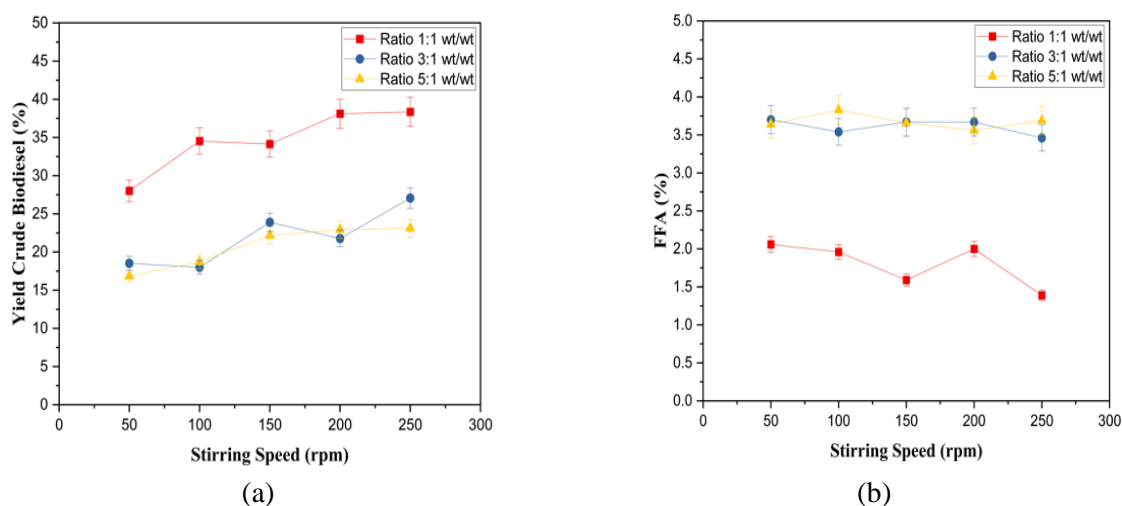


Figure 3 Effect of stirring speed (a) on %yield crude biodiesel based on the effect of CaO/Hydrotalcite loading ratio (b) on FFA crude biodiesel based on the effect of CaO/Hydrotalcite loading ratio

3.3.2. Effect of stirring rate and CaO loading on %FFA crude biodiesel

Figure 3b showed that the lowest FFA in loading ratio CaO/Hydrotalcite of 1:1 %wt/wt (250 rpm) was 1.39%. In addition, the higher stirring speed, the higher the level of decrease in %FFA. The results also showed that stirring rate had a significant effect on the FFA of crude biodiesel, where the higher stirring rate, the smaller the FFA observed. The acid number obtained affected the quality of biodiesel, and the higher the acid number in biodiesel, the lower its quality. This parameter could also affect the shelf life and the level of corrosiveness of the engine. The results showed that high acid numbers created deposits in the fuel system and reduced the quality of fuel system components.

3.4. Properties of *Nannochloropsis sp* Microalgae Biodiesel

Table 3 presented the physicochemical characteristics of *Nannochloropsis sp* microalgae biodiesel. The properties showed that *Nannochloropsis sp* microalgae could be used as biodiesel raw material. According to a previous study, biodiesel obtained from this species had a density quality of 0.839 g/m³ (Koech, Kumar, and Siagi, 2020; Salam, Velasquez-Orta, and Harvey, 2016). The results in Table 3 confirmed the good characteristics of *Nannochloropsis sp* microalgae for conversion into biodiesel.

Table 3 Physicochemical properties Characteristics of *Nannochloropsis sp* Microalgae Biodiesel

No	Properties	Test method	Standard	Values of algae Biodiesel
1	Density (g/cm ³)	SNI 7182:2015	0.850 – 0.890	0.869
2	Acid value (KOH/g)	ASTM D 664	max. 0.5	0.315
3	Free Fatty Acid value	-	max. 2	1.39

Based on the results of data processing of GC-MS, *Nannochloropsis sp* microalgae biodiesel using CaO/Hydrotalcite consisted of toluene (61.49%area) and Octane (38.51%), as shown in Table 4. Toluene was a hydrocarbon compound belonging to the olefin/alkenes group with a closed chain/aromatic type. Several studies had shown that it had the molecular formula of C₆H₅CH₃ and was a derivative of benzene compounds, namely methylbenzene. Meanwhile, octane was a hydrocarbon compound with an open/aliphatic chain type in the Alkane group. According to previous reports, it had the molecular formula of C₈H₁₈ was a compound belonging to the alkane group. Petroleum fractions were classified based on the amount of carbon in the hydrocarbon bond chain where C₄-C₁₂ was included in gasoline, C₁₀-C₁₆ was kerosene, and C₁₂-C₂₂ was categorized as biodiesel. Olefins contained in biofuels were C₇ hydrocarbons, and constituents were hydrocarbons with the number of carbon chains C₈ included in one of the gasoline, which had a range of carbon numbers C₄-C₁₂ (Salam, Velasquez-Orta, and Harvey, 2016).

Transesterification process in the presence of CaO occurred through various steps. Protons (H⁺) were extracted from CH₃OH at the CaO base site to form CH₃O⁻. In triglyceride compounds, the carbon atom of the carbonyl functional group was attacked by CH₃O⁻ to produce the alkoxy carbonyl compound, which was rearranged into FAME (the desired product) and the diglyceride anion. Subsequently, the diglyceride anion attacked the calcium hydroxide cation to form diglyceride and CaO, leading to the recovery of catalyst. Based on the results and previous studies, CaO/Hydrotalcite catalyst was a solution to obtain catalyst system that combined the characteristics of a bimodal pore distribution and micropores. In addition, it provided basic sites and high surface density along with the presence of a suitable macro or mesopore network responsible for triglyceride transport. This allowed rapid access to the active site from the bulk reaction medium and removal of products (glycerol and FAME) from catalyst (Nisar *et al.*, 2021). The reaction mechanism comprising the use of CaO is presented in Figure 4.

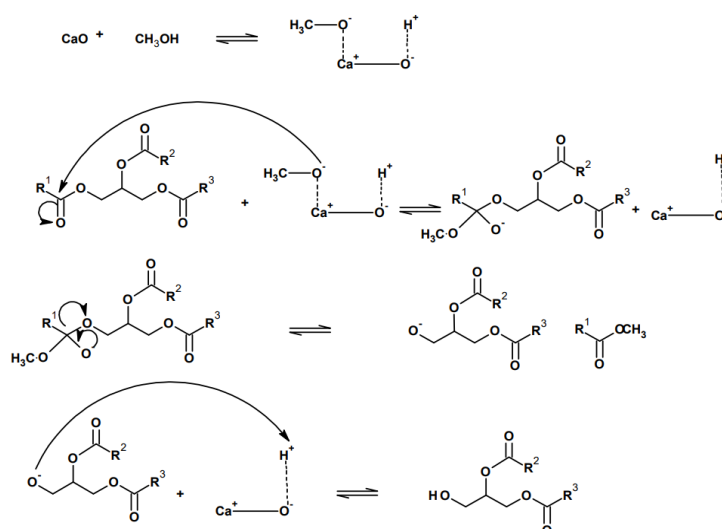
**Figure 4** A chemical reaction mechanism involving CaO

Table 4 *Nannochloropsis sp* microalgae biodiesel compounds using GC-MS analysis

No	Compounds	Retention Time	%area
1	Toluene	2.876	61.49
2	Octane	3.181	38.51

4. Conclusions

In conclusion, *Nannochloropsis sp* microalgae was subjected to in situ extraction-transesterification with methanol (solvent) and n-hexane (co-solvent) using CaO/Hydrotalcite catalyst. The highest yield obtained was 38.36%, with an FFA content of 1.39% (250 rpm; acid number 7.63 mg NaOH/g sample, and density 0.908 g/cm³). The GC-MS analysis showed the presence of toluene-like olefin (61.49%) and octane-like gasoline (38.51%)s group, showing the effectiveness of CaO/Hydrotalcite catalyst in converting *Nannochloropsis sp* microalgae to biodiesel using *in situ* stirring batch reactor transesterification.

Acknowledgments

The authors are grateful to the Ministry of Education, Culture, Research, and Technology for supporting this study in the Regular Fundamental Research Project. The number of contract was 183/E5/PG.02.00.PL/2023; 074/SP2H/PT/LL7/2023.

References

- Aisyah, A.N., Ni'maturrohmah, D., Putra, R., Ichsan, S., Kadja, G.T.M., Lestari, W.W., 2023. Nickel Supported on MIL-96(Al) as an Efficient Catalyst for Biodiesel and Green Diesel Production from Crude Palm Oil. *International Journal of Technology*, Volume 14(2), pp. 276–289
- Amusan, O.O., Louis, H., Hamzat, A.T., Oyebanji, O.F., Alagbe, A.T., Magu, T.O., 2019. Asian Journal of Nanoscience and Materials Synthesis and Characterization of CaO Catalyst Obtained from Achatina Achatina and Its Application in Biodiesel Production. *Asian Journal of Nanoscience and Materials*, Volume 2, pp. 271–277
- Asri, N.P., Saraswati, R., Hindarso, H., Suprpto, Mirzayanti, Y.W., Yogaswara, R.R., 2021. Study of Catalyst Support Utilization on Zno-Based Solid Catalyst to Its Activity at Transesterification of Kesambi (*Schleichera oleosa*) Oil. *IOP Conference Series: Materials Science and Engineering*, Volume 1034(1), p. 012059
- Budianto, A., Pambudi, W.S., Sumari, S., Yulianto, A., 2018. PID Control Design for Biofuel Furnace Using Arduino. *Telkomnika (Telecommunication Computing Electronics and Control)*, Volume 16(6), pp. 3016–3023
- Cercado, A.P.I., Ballesteros, F.C., Capareda, S.C., 2018. Biodiesel From Three Microalgae Transesterification Processes Using Different Homogenous Catalysts. *International Journal of Technology*, Volume 9(4), pp. 645–651
- Culaba, A.B., Ubando, A.T., Ching, P.M.L., Chen, W.H., Chang, J.S., 2020. Biofuel from Microalgae: Sustainable Pathways. *Sustainability*, Volume 12(19), pp. 1–19
- El-Sheekh, M.M., 2021. Biodiesel from Microalgae: Advantages and Future Prospective. *Egyptian Journal of Botany*, Volume 61(3), pp. 669–671
- Ghedini, E., Taghavi, S., Menegazzo, F., Signoretto, M., 2021. A Review on The Efficient Catalysts for Algae Transesterification to Biodiesel. *Sustainability*, Volume 13(18), p. 10479

- Kanagesan, K., Palanisamy, K.M., Maniam, G.P., Hasbi, M., Rahim, A., Govindan, N., 2020. Biodiesel Production by Microalgae *Nannochloropsis sp*. Grown in Palm Oil Mill Effluent. *Maejo International Journal of Energy and Enviromental Communication*, Volume 2(3), pp. 21–26
- Kazemifard, S., Nayebzadeh, H., Saghatoleslami, N., Safakish, E., 2019. Application of Magnetic Alumina-Ferric Oxide Nanocatalyst Supported by KOH for in-situ Transesterification of Microalgae Cultivated in Wastewater Medium. *Biomass and Bioenergy*, Volume 129, p. 105338
- Koech, A.K., Kumar, A., Siagi, Z.O., 2020. In Situ Transesterification of Spirulina Microalgae to Produce Biodiesel Using Microwave Irradiation. *Journal of Energy*, Volume 2020, pp. 1–10
- Larida, A.Q., Bañaga, J.B., 2021. Extraction and Characterization of Algal Oil from Lake Sebu, South Cotabato: A Potential Source of Biodiesel. *Journal of Science and Science Education*, Volume 5(1), pp. 12–25
- Lomeu, A.A., Mendonça, H.V.de, Mendes, M.F., 2023. Microalgae As Raw Material for Biodiesel Production: Perspectives and Challenges of The Third Generation Chain. *Engenharia Agrícola*, Volume 2023, p. 43
- Ma, X.N., Chen, T.P., Yang, B., Liu, J., Chen, F., 2016. Lipid production from *Nannochloropsis*. *Marine Drugs*, Volume 14(4). p. 61
- Makareviciene, V., Sendzikiene, E., 2022. Application of Microalgae Biomass for Biodiesel Fuel Production. *Energies*, Volume 15(11), p. 4178
- Manurung, R., Parinduri, S.Z.D.M., Hasibuan, R., Tarigan, B.H., Siregar, A.G.A., 2023. Synthesis of Nano-CaO Catalyst with SiO₂ Matrix Based on Palm Shell ash as Catalyst Support for One Cycle Developed in The Palm Biodiesel Process. *Case Studies in Chemical and Environmental Engineering*, Volume 7, p. 100345
- Marlinda, L., Prajitno, D.H., Roesyadi, A., Gunardi, I., Mirzayanti, Y.W., Al-Muttaqii, M., Budianto, A., 2022. Biofuel From Hydrocracking of Cerbera Mangas Oil Over Ni-Zn/HZSM-5 Catalyst. *Ecletica Quimica*, Volume 47(1), pp. 17–39
- Medipally, S.R., Yusoff, F.M., Banerjee, S., Shariff, M., 2015. Microalgae as Sustainable Renewable Energy Feedstock for Biofuel Production. *BioMed Research International*, Volume 2015, p. 519513
- Mirzayanti, Y.W., Alisa, A., Sari, D., 2020a. Biodiesel Production from Rice Bran using a Two-Stage In-Situ Method Using Sulfuric Acid and CaO/Hydrotalcite Catalysts. *Jurnal Rekayasa Mesin*, Volume 11(3), pp. 375–382
- Mirzayanti, Y.W., Kurniawansyah, F., Prajitno, D.H., Roesyadi, A., 2020b. Selectivity of Zinc-Molybdenum Catalyst to Produce Gasoil Via Catalytic Hydrocracking of Kapook Seed Oil (*Ceiba Pentandra*). *Journal of Physics: Conference Series*, Volume 1442, p. 012055
- Mohamad, M., Ngadi, N., Wong, S., Yahya, N.Y., Inuwa, I.M., Lani, N.S., 2018. Synthesis and Characterization of CaO-TiO₂ for Transesterification of Vegetable Palm Oil. *International Journal of Engineering, Transactions B: Applications*, Volume 31(8), pp. 1326–1333
- Navajas, A., Campo, I., Moral, A., Echave, J., Sanz, O., Montes, M., Odriozola, J.A., Arzamendi, G., Gandía, L.M., 2018. Outstanding Performance of Rehydrated Mg-Al Hydrotalcites as Heterogeneous Methanolysis Catalysts for The Synthesis of Biodiesel. *Fuel*, Volume 211, pp. 173–181
- Nisar, S., Hanif, M. A., Rashid, U., Hanif, A., Akhtar, M.N., Ngamcharussrivichai, C., 2021). Trends in Widely Used Catalysts for Fatty Acid Methyl Esters (FAME) Production: A Review. *Catalysts*, Volume 11(9), p. 1085

- Purkan, P., Safa, A., Abdulloh, A., Baktir, A., Arissirajudin, R., Hermansyah, H., Kim, S.W., 2021. In-Situ Biodiesel Synthesis from Microalgae *Nannochloropsis Oculata* Using NiO Nanocatalyst. *Rasayan Journal of Chemistry*, Volume 14(3), pp. 2097–2103
- Rahma, F.N., Hidayat, A., 2023. Biodiesel Production from Free Fatty Acid using ZrO₂/Bagasse Fly Ash Catalyst. *International Journal of Technology*, Volume 14(1), pp. 206–218
- Salam, K.A., Velasquez-Orta, S.B., Harvey, A.P., 2016. A Sustainable Integrated in Situ Transesterification of Microalgae for Biodiesel Production and Associated Co-products - A Review. *Renewable and Sustainable Energy Reviews*, Volume 65, pp. 1179–1198
- Salam, K.A., Velasquez-Orta, S.B., Harvey, A.P., 2016. Surfactant-Assisted Direct Biodiesel Production from Wet *Nannochloropsis Oculata* by in Situ Transesterification/Reactive Extraction. *Biofuel Research Journal*, Volume 3(1), pp. 366–371
- Sardi, B., Ningrum, R.F., Ardiansyah, V.A., Qadariyah, L., Mahfud, M., 2022. Production of Liquid Biofuels from Microalgae *Chlorella sp.* via Catalytic Slow Pyrolysis. *International Journal of Technology*, Volume 13(1), pp. 147–156
- Shirazi, Y., Viamajala, S., Varanasi, S., 2020. In situ and Ex situ Catalytic Pyrolysis of Microalgae and Integration with Pyrolytic Fractionation. *Frontiers in Chemistry*, Volume 8
- Tabatabaei, M., Aghbashlo, M., Dehghani, M., Panahi, H.K.S., Mollahosseini, A., Hosseini, M., Soufiyan, M.M., 2019. Reactor Technologies for Biodiesel Production and Processing: A Review. *Progress in Energy and Combustion Science*, Volume 74, pp. 239–303
- Wahyono, Y., Hadiyanto, Budihardjo, M.A., Hariyono, Y., Baihaqi, R.A., 2022. Multifeedstock Biodiesel Production from a Blend of Five Oils through Transesterification with Variation of Moles Ratio of Oil: Methanol. *International Journal of Technology*, Volume 13(3), pp. 606–618
- Win, T.T., Khine, M.M., 2017. Synthesis and Characterization of CaO and KF Doped CaO (KF/CaO) Derived from Chicken Eggshell Waste as Heterogeneous Catalyst in Biodiesel Production. *Technology, and Sciences (ASRJETS) American Scientific Research Journal for Engineering*, Volume 38(2), pp. 134–151
- Wood, D.A., 2021. Microalgae to Biodiesel - Review of Recent Progress. *Bioresource Technology Reports*, Volume 14, p. 10065
- Zhang, C.Y., Shao, W.L., Zhou, W.X., Liu, Y., Han, Y.Y., Zheng, Y., Liu, Y.J., 2019. Biodiesel Production by Esterification Reaction on K⁺ Modified MgAl-Hydrotalcite Catalysts. *Catalysts*, Volume 9(9), p. 742
- Zul, N.A., Ganesan, S., Hamidon, T.S., Oh, W.-Da, Hussin, M.H., 2021. A Review on The Utilization of Calcium Oxide as a Base Catalyst in Biodiesel Production. *Journal of Environmental Chemical Engineering*, Volume 9(4), p. 105741