pISSN 2302-1616, eISSN 2580-2909
Vol 12, No. 2, December 2024, pp. 92-100
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v12i2.55910

Lipid from microalgae isolated from Cengklik Reservoir, Boyolali, Central Java for prospective biodiesel sources

Siti Lusi Arum Sari[1*], Suratman[1], Sunarto[2], Tjahjadi Purwoko[1], Dewi Larasati[1], Stefanus Junliem Nugratama Herina[1]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret
Jl. Ir. Sutami 36A, Surakarta, Central Java, Indonesia. 57126
[2]Department of Environmental Science, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret
Jl. Ir. Sutami 36A, Surakarta, Central Java, Indonesia. 57126
*Email: sitilusi@staff.uns.ac.id

ABSTRACT. The availability and sustainability of the algae-based biodiesel industry depend on the adequate selection of microalgae strains. Based on this, it is important to explore local microalgae from Indonesia as a feedstock for biodiesel. This study aimed to isolate and screen freshwater microalgae with lipid-producing abilities. A total of three microalgal isolates have been successfully isolated from the Cengklik Reservoir (-7.506712, 110.723582). Based on the cell morphology, the three isolates were identified as *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp. Nile red staining indicated that the three microalgal isolates *contained* neutral lipid droplets in their cells, as shown by the presence of a yellow or orange luminescence under a fluorescence microscope. The results of this study indicated that *Scenedesmus* sp. and *Oscillatoria* sp. had more potential as feedstocks for biofuels due to their higher lipid content and productivity. This research succeeded in obtaining microalgae isolates from Cengklik reservoir, Boyolali, Central Java which can be used as a source of lipids for biodiesel.

Keywords: biodiesel; *Chlorella* sp.; lipid production; microalgae; *Oscillatoria* sp.; *Scenedesmus* sp.

Article History: Received 5 March 2025; Received in revised form 22 July 2025; Accepted 22 July 2025; Available online 2 August 2025.
How to Cite This Article: Sari SLA, Suratman S, Sunarto S, Purwoko T, Larasati D, Herina SJN. 2024. Lipid from microalgae isolated from Cengklik Reservoir, Boyolali, Central Java for prospective biodiesel sources. Biogenesis: Jurnal Ilmiah Biologi. vol 12(2): 92-100. doi: https://doi.org/10.24252/bio.v12i2.55910.

INTRODUCTION

Energy is the main driver of economic growth and plays an important role in the sustainability of modern economies and societies. According to the Executive summary – World Energy Outlook (2022), the proportion of fossil fuels in the global energy structure has remained high for many decades, about 80%. Fossil fuels are non-renewable fuels and cause various negative impacts on the environment. It has been shown that 98% of carbon emissions results from fossil fuels (Gashaw *et al*., 2015). The trend of energy development is to achieve carbon peaking and carbon neutrality, reduce carbon dioxide emissions, and solve the problem of global climate change (Zhang *et al*., 2022). This has encouraged the development of alternative renewable fuels, which are more environmentally friendly and produce less CO_2 emissions. These alternative fuels include bioethanol and biodiesel which can be produced from biomass or waste feedstocks, renewable, and a significantly reduced contribution to global warming (Casanova *et al*., 2023)

Biodiesel, commonly known as fatty acid methyl esters (FAME), is synthesized through esterification or transesterification from edible or non-edible oils (Zhang *et al*., 2022). Biodiesel can be produced through the transesterification reaction of any natural oil or fat using short-chain alcohols such as methanol and ethanol, which are responsible for providing methyl and ethyl radicals, respectively, and a base or acid catalyst (Nunes *et al*., 2021). Biodiesel has the potential to be developed as an alternative fuel due to its ability to adapt to gasoline for a maximum mixture of 85% without the need for any engine modifications (Hossain *et al*., 2019).

First-generation biodiesel production uses raw materials from food crops, edible oils, and animal fat. First generation biodiesel is synthesized through esterification of vegetable oil and ethanol which is generated primarily from food crops (92% is from corn and sugar cane) (Vignesh *et al*., 2021). The advantages of first generation biodiesel are the sustainability of the raw materials and the easy conversion process (Singh *et al*., 2024). However, the use of food crops as biodiesel row materials is

hampered by the food security issues, high production costs, and limited cultivation space (Asase *et al*., 2024). Second-generation biofuel production uses non-edible raw materials such committed energy crops (for example, jatropha, enduring grasses, short turn coppice, and other lignocellulosic plants), squander biomass (for example, agrarian and ranger service buildup), and waste materials (for example squander cooking oil) (Malode *et al*., 2022). The production of bioethanol using these materials would no longer need land and would thus have no impact on the production of food and fibre crops (Vignesh *et al*., 2021). However, in general non-edible crops are planted on marginal land resulting in low crop yields. Planting non-edible crops on agricultural land will increase the yield, but there is a significant impact on both food production and social economy (Singh *et al*., 2024). Third-generation biodiesel production uses oil from microalgae and waste oils. Compared to 1st and 2nd generations, microalgae are considered to have more potential because of no competition for land or food markets with crops, lower greenhouse effect, better growth rate and productivity, and higher oil content (Singh *et al*., 2024). Another advantage is that algae oil is easily processed into diesel and gasoline, and certain algae species can be genetically modified so that carbon metabolism pathways facilitate the production of the required final products (Neto *et al*., 2019). Microalgae use neutral lipids (acylglycerols, mostly triacyl-glycerols (TAG) for energy storage (Casanova *et al*., 2023).

Indonesia, as a country with great biodiversity, certainly has abundant biological potential. Research on microalgae biodiversity in Indonesia has been widely reported, including in java (Santosa, 2010; Hariyati & Putro, 2019; Zakiyah *et al*., 2020; Mahmudi *et al*., 2023), Sumatra (Pane *et al*., 2023), Bali (Yudana *et al*., 2021), and Kalimantan (Kushadiwijayanto *et al*., 2024). As many as 1,058 species, 225 genera, 130 families and 23 classes of microalgae can be found throughout Indonesia (Andriani *et al*., 2024). This microalgae has the potential to be used in various fields, including as functional food ingredient (Nur, 2014; Wahyuningtyas *et al*., 2024), metabolite producer (Noerdjito *et al*., 2024), bioindicator (Hariyati & Putro, 2019), heavy metal biosorbent (Rinanti *et al*., 2021), and, of course, have the potential to be developed as a source of oil for biodiesel production (Jumiarni & Anggraini, 2021). Various studies showed that Indonesian waters were a source of lipid-producing microalgae for biodiesel (Santosa, 2010; Saputro *et al*., 2019). The most important criteria for the selection of microalgae species for the development of a competitive biodiesel production process are the ability of the microalgae to accumulate lipids and grow rapidly, and adapt to the local environment (Jazzar *et al*., 2015). Native microalgae isolated from around the production area will adapt more easily to local conditions so that they can support high productivity (Gour et al., 2014).

Based on this, it is important to explore local microalgae as a feedstock for biodiesel production. This study aimed to isolate and screen freshwater microalgae with lipid-producing abilities. The findings have significant implications for advancing sustainable energy strategies, particularly in regions rich in aquatic biodiversity. By identifying indigenous microalgae strains with high lipid content, this research supports the development of locally sourced biofuels, reducing dependence on imported fossil fuels and enhancing energy security. Moreover, integrating microalgae-based biodiesel into existing energy frameworks could contribute to lowering greenhouse gas emissions and promoting circular bioeconomy practices.

MATERIALS AND METHODS

Study area. Water samples were taken from the Cengklik Reservoir, located in Ngargorejo, Ngemplak, Boyolali, Central Java, Indonesia. Its coordinates were -7.506712 and 110.723582 (Fig. 1). A total of 10 L of water was taken and then filtered through a plankton net until approximately 10 mL of water was obtained.

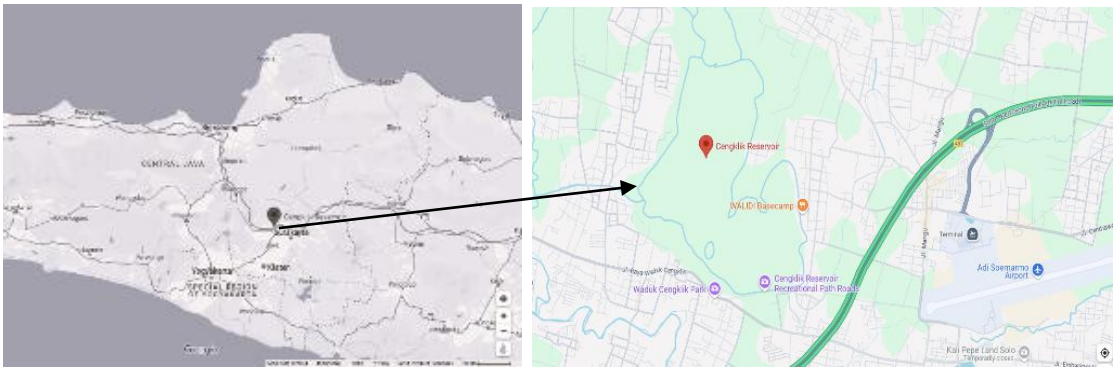

Fig. 1. Location of Cengklik Reservoir in Ngargorejo, Ngemplak, Boyolali, Central Java, Indonesia

Media. The media for microalgae growth was Bold's Basal Medium (BBM) with the composition of (mg/L): $NaNO_3$ (250), $MgSO_47H_2O$ (75), NaCl (25), K_2HPO_4 (75), KH_2PO_4 (175), $CaCl_2$ $2H_2O$ (25), $ZnSO_4 \cdot 7H_2O$ (8.82), $MnCl_2 \cdot 4H_2O$ (1.44), MoO_3 (0.71), CuSO4·5H2O (1.57), $Co(NO_3)_2 \cdot 6H2O$ (0.49), H_3BO_3 (11.42), Na_2EDTA (50), KOH (31), $FeSO_4 \cdot 7H_2O$ (4.98) (De Lourdes *et al*., 2017).

Isolation of microalgae. Microalgae isolation was carried out using the spread plate method according to the method by Lee *et al*. (2014), with modifications. A total of 0.1 mL of water sample was inoculated on Bold's Basal Agar Medium (BBA) by the spread plate method. Each sample at each station was planted in three petri dishes (triplo). Incubation was carried out using light-emitting diode (LED) lamps with a light intensity of 1500 Lux continuously for 24 hours. Incubation was carried out for three weeks. Separated colonies were purified using the quadrant method. Pure microalgal isolates were then grown in liquid BBM for further research.

Identification of microalgae. Morphological identification was carried out by observation under a light microscope. The characteristics observed were cell organization and shape, the presence or absence of flagella, and typical color related to the type of pigment. The reference used for microalgae identification was Bellinger & Sigee (2015).

Neutral lipid determination. Neutral lipid content was indicated by yellow or orange luminescence under the microscope after Nile red (9-diethylamino-5Hbenzo [α] phenoxazine-5-one, Sigma–Aldrich) staining. Nile red staining was carried out based on the method described by Cooksey *et al*. (1987). Nile red solution was made by dissolving 1 mg of Nile red in 1 mL of acetone (Pan *et al*., 2011). One loop of pure microalgal culture from BBA was inoculated into 3 mL of liquid BBM. Cultures were incubated for 16 days at a temperature of 25 ± 2°C, a light intensity of 2000 lux, and an exposure time of 18 hours (Pachiappan *et al*., 2015). Cultivation was carried out in 3 replications. A total of 10 µL of Nile Red solution was dropped onto 1 mL of microalgae culture, homogenized by vortexing, and incubated for 20-30 minutes. After Nile red staining, microalgae were observed under a fluorescence microscope with a blue excitation filter of 450-490 nm. Microalgae that contain neutral lipids showed a shiny yellow or orange luminescence.

Measurement of microalgal growth. A total of 10 mL of culture from liquid BBM aged 16 days was inoculated into 90 mL of liquid BBM. So, the total volume for experiment was 100 mL.Cultures were incubated at a temperature of 25 ± 2°C, light intensity of 2000 lux, exposure time of 18 hours (Pachiappan *et al*., 2015), and aeration. The measurement of microalgal growth was carried out by counting the number of cells every 24 hours. A total of 100 µL of culture was dropped into a hemacytometer then observed under a light microscope, and the number of cells was counted. The number of cells was determined using the following formula:

$$\text{Number of cells/mL} = \frac{\text{number of cells x dilution}}{\text{number of plots x volume (mL)}}$$

Measurement of biomas and lipid production from microalgae. A total of 10 mL of culture from liquid BBM aged 16 days was inoculated into 90 mL of liquid BBM. Cultures were incubated

at a temperature of 25 ± 2°C, light intensity of 2000 lux, exposure time of 18 hours (Pachiappan *et al*., 2015), and aeration. Incubation was carried out until the peak of the logarithmic phase according to the results of previous growth measurements. Microalgae cultivation was carried out in 3 replications. The microalgae culture was centrifuged at 4000 rpm 25°C for 10 minutes. The supernatant was discarded, and the pellet was dried by lyophilization. The microalgal biomass was weighed to determine the dry weight, then extracted to determine the total lipid content.

Lipid extraction from microalgae. Lipid extraction from microalgae was carried out according to the method of Bligh & Dyer (1959), with modifications. Lipid was extracted with the solvents chloroform:methanol:water (1:2:0.8 v/v).The solvent was added to the dried microalgae, then incubated in a water bath shaker for 24 hours at 27°C. After 24 hours, the biomass suspension was added with a solvent until the ratio of chloroform:methanol:water became 2:2:1.8 v/v, vortexed, and then centrifuged at 4000 rpm for 25 minutes. The lower layer was removed and evaporated using a fume hood. The total lipid weight was determined gravimetrically. Total lipid content was determined using the following formula:

$$\text{Total lipid contet (\%)} = \frac{\text{lipid weight (g)}}{\text{Biomass dy weight (g)}}\ x\ 100\%$$

Lipid productivity was determined using the following formula:

Lipid productivity (g/L) = Biomass Productivity (g dry weight/L) × Total Lipid Content (%)

Data analysis. The biomass and lipid productivity datas obtained were analyzed via one-way analysis of variance (ANOVA) at α 5% ($p<0.05$). If there was a significant difference, the analysis was continued with the Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

Isolation and identification of microalgae. A total of three microalgal isolates have been successfully isolated from water samples taken from the Cengklik Reservoir, Boyolali, Central Java, Indonesia (-7.506712, 110.723582). Based on the morphological characteristics of the cells, the three isolates were identified as *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp. respectively. *Chlorella* sp. cells were green, round in shape, had cup-shaped protoplasm, without spines and flagella, and vary in diameters, namely between 4.6-7.1 µm (Fig. 2A & 2B). *Scenedesmu*s sp. had cylindrical cell shape tapered ends, the outer cells were crescent-shaped, the ends of the cells are without accessories or spines, without fagella, green in color, vary in size between 5.8-28 µm in length and 4.3-14.2 µm in width, and form colonies of 2 or 4 cells (Fig. 2C & 2D). The *Oscillatoria* sp. cell shape was an unbranched cylindrical filament, usually bluish-green in color, no flagella, and vary in size, namely between 1.3-2.14 µm in width and 7.3-130 µm in length (Fig. 2E & 2F).

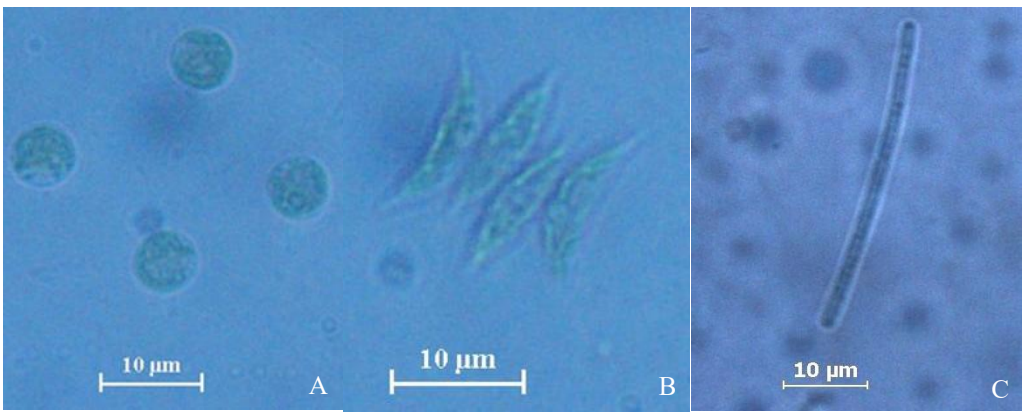

Fig. 2. *Chlorella* sp. (A), *Scenedesmus* sp. (B), and *Oscillatoria* sp. (C) isolated from Cengklik Reservoir under light microscope with a magnification of 1000x

Neutral lipid determination. The results of observations under a light microscope after staining with Nile red can be seen in Fig. 3. The yellow luminescence showed the neutral lipid droplet. The results of this research indicated that *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp have been isolated from the Cengklik reservoir contain neutral lipid granules in their cells.

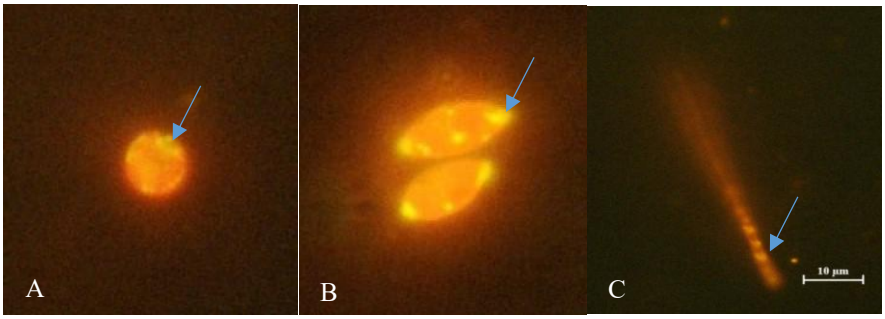

Fig. 3. *Chlorella* sp. (A), *Scenedesmus* sp. (B), and *Oscillatoria* sp. (C) under a fluorescence microscope with a blue excitation filter of 450-490 nm after staining by Nile Red. The yellow fluorescence (arrow sign) showed the neutral lipid droplet

Microalgae growth. The growth curves (Figure 4.) showed that *Chlorella* sp. and *Scenedesmus* sp. reached their peak of logarithmic phase on 12 days incubation period, while *Oscillatoria* sp. on 9-13 days incubation period. Based on this, for biomass and lipid production, microalgae were cultivated for 12 days.

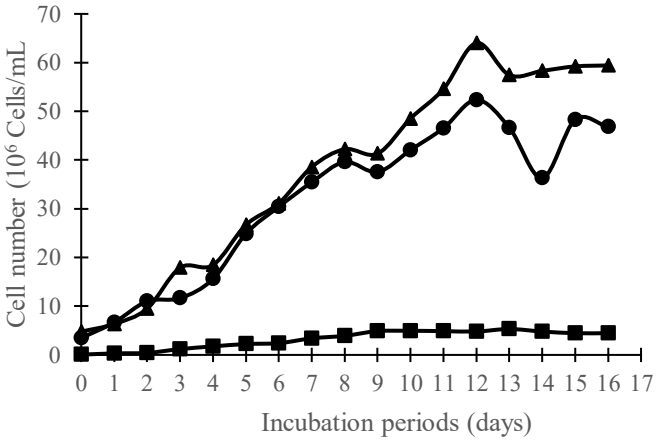

Fig. 4. *Chlorella* sp. (-●-), *Scenedesmus* sp. (-▲-), and *Oscillatoria* sp.(-■-) growth curves on liquid Bold Basal Medium (BBM). Cultures were incubated at a temperature of 25 ± 2°C, light intensity of 2000 lux, exposure time of 18 hours

Biomass and lipid production. The biomass productivity of three microalgal isolates did not differ significantly with a yield of approximately 1 g/L (Table 1). The lowest lipid productivity was shown by *Chlorella* sp., whereas *Scenedesmus* sp., and *Oscillatoria* sp. did not show significant differences.

Table 1. Biomass and lipid productivity of *Chlorella* sp, *Scenedesmus* sp., and *Oscillatoria* sp. on Bold's Basal Medium

Microalgae	Biomass productivity (g dry weight/L)	Total Lipid content (%)	Lipid productivity (g/L)
Scenedesmus sp.	1.13 ± 0.32[a]	28,12 ± 9.45[b]	0.30 ± 0.06[b]
Chlorella sp.	1.09 ± 0.56[a]	14.21± 3.10[a]	0.14± 0.04[a]
Oscillatoria sp.	1.00 ± 0.12[a]	21,92 ± 4.25[ab]	0.22 ± 0.07[ab]

Notes: numbers followed by different letters in one column indicate significant differences at α 5% ($p<0.05$)

The present study successfully isolated and identified three distinct microalgal isolates, *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp., from water samples collected from the Cengklik Reservoir

in Boyolali, Central Java, Indonesia.According to Bellinger & Sigee (2015), *Chlorella* cells (2–10 µm diameter) are spherical to sub-spherical with one parietal chloroplast nearly filling the cell, and having one pyrenoid. *Chlorella* cells that have been successfully isolated in this study were similar to *Chlorella* cells which was isolated by Rahman *et al*. (2019), namely: round or spherical, solitaire, thick cell walls, pyrenoid inside the u-shape chloroplast with light green to yellowish color. *Oscillatoria* cells were similar to *Oscillatoria* cells which were described by Bellinger & Sigee (2015), cell shape was straight or curved with tips that may be rounded or specially shaped, single or in groups, free-floating or attached, short or quite long, blue-green, olive green, reddish or brownish in colour. The *Oscillatoria* filaments were varying in sizes, from 57.60µm to 69.05 µm in length and from 5.20µm to 9.55µm in width (Rani *et al*., 2016), and sometimes purple when they were old (Mühlsteinova *et al*., 2018). The *Scenedesmus* isolates had similar morphological characters with S. *dimorphus* which were described by Bellinger & Sigee (2015), namely: had cylindrical cells with tapered ends, the outer cells were crescent-shaped, and vary in size between 5.8-28 µm in length and 4.3-14.2 µm in width. The successful isolation of these diverse microalgae from the Cengklik Reservoir highlights its potential as a source for novel or locally adapted strains with biotechnological relevance.

A critical aspect of microalgae's biotechnological utility is their ability to accumulate neutral lipids, which are precursors for various valuable products, including biofuels. Only neutral lipid fraction can be used to produce biofuels (Dancs *et al*., 2023). The Nile Red staining confirmed the presence of neutral lipid droplets within the cells of all three microalgal isolates: *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp. While it's known that microalgal cells naturally contain lipids, the significant yellow fluorescence observed indicated a notable accumulation of these neutral lipids and can be used as a quick investigated tool for screening hyper-lipid producing microalgae cells (Arathi *et al*., 2020). Several previous studies also used Nile red staining to detect the presence of neutral lipids in microalgae cells. Sharma *et al*. (2019) used Nile red as a staining dye to investigated for lipid content qualitatively in microalgal consortia. Acording to Priyanka *et al*. (2020), Nile Red protocol was a robust, simple and cost-effective method for quantifying neutral lipids in *Chlorella emersonii* and *Pseudokirchneriella subcapitata*.

Selecting microalgae species with a high lipid content is one of the key processes in the algal biofuel production system. The results of this study indicated that the biomass productivity of three microalgal isolates did not differ significantly; however, their lipid productivity differed considerably. *Chlorella* sp. displayed the lowest lipid productivity, while *Oscillatoria* sp. and *Scenedesmus* sp. did not differ significantly. Previous research conducted by Mathimani *et al*. (2021) showed that the lipid content and lipid productivity of *Scenedesmus* sp. was higher than *Chlorella* sp. Commercial production of biodiesel from microalgae requires fast growth microalgae with high biomass and lipid productivity. The biomass productivity and lipid content of *Scenedesmus* sp. in Bold's Basal Medium were 300 ± 60 mg/L and 28.12 ± 9.4%, respectively. The findings of this study were almost the same as the results of the study by Zhang *et al*. (2015) which used domestic secondary effluent (DSE) as a medium for S. *dimorphus* for lipid production with lipid productivity and lipid content of 244 mg L−1 and 26.06%, respectively. The results of this study indicated that the *Oscillatoria* sp. and *Scenedesmus* sp. were more potential as a feedstock for biofuels due to those higher yield in terms of lipid content and productivity. *Scenedesmus* has good potential to be developed as a biodiesel raw material because its productivity (Trivedi *et al*., 2022; Anand *et al*., 2023; Nicodemou *et al*., 2024; Ogbonna *et al*., 2024). *Scenedesmus* can be grown using various types of municipal wastewater for pollutant removal and production of lipid so it is more economical and environmentally friendly (Silambarasan *et al*., 2023). Compared to *Scenedesmus*, information about *Oscallitoria* as a biodiesel raw material is still quite limited. However, *Oscillatoria* can also be grown using wastewater (Fatah *et al*., 2020).

During this research, qualitative and quantitative screening was carried out in stages. Qualitative screening was carried out by observing cells under a fluorescence microscope after NR staining and

then quantitative screening was carried out by measuring biomass and lipid production. This 2-stage screening is time consuming so it cannot detect the lipid content in the culture near real time. Future research can use the method developed by Natunen (2020). Nile Red can penetrates the microalgal cell walls and stains the intracellular neutral lipids. Thus, the neutral lipid content in the culture can be measured near real time by measuring the fluorescence after addition of NR to the culture sample.

This research succeeded in obtaining microalgae isolates which can be used as a source of lipids for biodiesel. However, its use is faced with various challenges. One of the disadvantages of using microalgae is the high economic costs due to low productivity, so further research is needed to increase the biomass and lipid content in microalgae cells. Strategies that can be developed include: modulating light intensity in culture, controlling and varying CO_2 levels and temperature, inducing nutrient starvation in culture, applying stress by introducing heavy metals or inducing high salinity conditions, applying nanotechnology and genetic engineering (Natunen, 2020).

CONCLUSION

This research succeeded in obtaining microalgae isolates, namely: *Chlorella* sp., *Scenedesmus* sp., and *Oscillatoria* sp., which can be used as a source of lipids for biodiesel. The three microalgal isolates contained neutral lipid droplets in their cells. The three microalgal isolates had the same biomass productivity but *Scenedesmus* sp. and *Oscillatoria* sp. had greater lipid productivity. However, further research is needed to increase the biomass and lipid content in microalgae cells to reduce production costs, including: modulating culture condition, applying stress, nanotechnology and genetic engineering.

ACKNOWLEDGEMENTS

Authors thank to Universitas Sebelas Maret, Surakarta, Indonesia for financial support through the Research Group Research Grant in 2024 (Contract number: 194.2/UN27.22/PT.0l.03/2024).

REFERENCES

Anand V, Kashyap M, Bala K. 2023. Impact of H_2O_2 on biomass and lipid accumulation in *Scenedesmus* sp.: Promising species for biofuel production. *Biocatalysis and Agricultural Biotechnology*. vol 51(102795). doi: https://doi.org/10.1016/j.bcab.2023.102795.

Andriani F, Hendrayanti D, Yasman Y. 2024. Exploring microalgae diversity in Indonesia: Harnessing potential for lead bioremediation. *Bioeduscience*. vol 8(1): 26–42. doi: https://doi.org/10.22236/jbes/13225.

Arathi S, Kumar JT, Jothibasu K, Karthikeyan S, Suchitra R. 2020. Qualitative and quantitative estimation of algal lipids for biofuel production. *International Journal of Chemical Studies*. vol 8(4): 2451–2459. doi: https://doi.org/10.22271/chemi.2020.v8.i4ab.10003.

Asase RV, Okechukwu QN, Ivantsova MN. 2024. Biofuels: Present and future. *Environment, Development and Sustainability*. doi: https://doi.org/10.1007/s10668-024-04992-w.

Bellinger EG, Sigee DC. 2015. *Freshwater algae: Identification, enumeration and use as bioindicators*. 2nd ed. Wiley-Blackwell.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*. vol 37(8): 911–917. doi: https://doi.org/10.1139/o59-099.

Casanova LM, Mendes LBB, Corrêa TdeS, da Silva RB, Joao RR, Macrae A, Vermelho AB. 2023. Development of microalgae biodiesel: Current status and perspectives. *Microorganisms*. vol 11(1): 1-120. doi: https://doi.org/10.3390/microorganisms11010034.

Chen Z, Wang L, Qiu S, Ge S. 2018. Determination of microalgal lipid content and fatty acid for biofuel production. *BioMed Research International*. vol 2018(1503126): 1-17. doi: https://doi.org/10.1155/2018/1503126.

Cooksey KE, Guckert JB, Williams SA, Callis PR. 1987. Fluorometric determination of the neutral lipid content of microalgal cells using Nile Red. *Journal of Microbiological Methods*. vol 6: 333–345. doi: https://doi.org/10.1016/0167-7012(87)90019-4.

Dancs G, Kakucska G, Dobrányi S, Ecker J, Fülöp L. 2023. Efficient method for the determination of the neutral lipid content of oil-producing microalgae strains required for biodiesel. *Fuel*. vol 331(2). doi: https://doi.org/10.1016/j.fuel.2022.125831.

De Lourdes FMM, Josefina RRMD, Ulises MMC, De Jesús MRA. 2017. Tolerance and nutrients consumption of *Chlorella vulgaris* growing in mineral medium and real wastewater under laboratory conditions. *Open Agriculture*. vol 2(1): 394–400. doi: https://doi.org/10.1515/opag-2017-0042.

Executive summary – World Energy Outlook 2022 – Analysis – IEA. 2022. Retrieved 10 October 2023, from https://www.iea.org/reports/world-energy-outlook-2022/executive-summary.

Gashaw A, Getachew T, Teshita A. 2015. A review on biodiesel production as alternative fuel. *Journal of Forest Product and Industries*. vol 4(2): 2325–4513.

Gour RS, Kant A, Chauhan RS. 2014. Screening of microalgae for growth and lipid accumulation properties. *Journal of Algal Biomass Utilization*. vol 5(1): 38–46.

Hariyati R, Putro SP. 2019. Bioindicator for environmental water quality based on saprobic and diversity indices of planktonic microalgae: A study case at Rawapening Lake, Semarang District, Central Java, Indonesia. *Journal of Physics: Conference Series*. vol 1217(1): 1-7. doi: https://doi.org/10.1088/1742-6596/1217/1/012130.

Hossain N, Mahlia TMI, Saidur R. 2019. Latest development in microalgae-biofuel production with nano-additives. *Biotechnology for Biofuels*. vol 12(125): 1-16. doi: https://doi.org/10.1186/s13068-019-1465-0.

Jazzar S, Quesada-Medina J, Olivares-Carrillo P, Marzouki MN, Acién-Fernández FG, Fernández-Sevilla JM, Molina-Grima E, Smaali I. 2015. A whole biodiesel conversion process combining isolation, cultivation and in situ supercritical methanol transesterification of native microalgae. *Bioresource Technology*. vol 190: 281–288. doi: https://doi.org/10.1016/j.biortech.2015.04.097.

Jumiarni D, Anggraini N. 2021. Characterization of microalgae from lowlands in South Sumatera (Indonesia) as a potential source for biodiesel production. *Journal of Physics: Conference Series*. vol 1731(012002): 1-7. doi: https://doi.org/10.1088/1742-6596/1731/1/012002.

Kushadiwijayanto IA, Sofiana AA, Nguyen MS, Adjout DH. 2024. Investigating species composition and abundance of marine microalgae from Temajo Island waters. *Jurnal Biodjati*. vol 9(2): 202–217. doi: https://doi.org/10.15575/biodjati.v9i2.380286.

Lee K, Eisterhold ML, Rindi F, Palanisami S, Nam PK. 2014. Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources. *Journal of Natural Science, Biology and Medicine*. vol 5(2): 333–339. doi: https://doi.org/10.4103/0976-9668.136178.

Mahmudi M, Arsad S, Lusiana ED, Musa M, Fitrianesia F, Ramadhan SF, Arif AR, Savitri FR, Dewinta AA, Ongkosongo AD. 2023. Microalgae diversity in varying habitat characteristics in Pasuruan and Sidoarjo coastal areas, East Java, Indonesia. *Biodiversitas*. vol 24(8): 4418–4426. doi: https://doi.org/10.13057/biodiv/d240823.

Malode SJ, Gaddi SAM, Kamble PJ, Nalwad AA, Muddapur UM, Shetti NP. 2022. Recent evolutionary trends in the production of biofuels. *Materials Science for Energy Technologies*. vol 5: 262–277. doi: https://doi.org/10.1016/j.mset.2022.04.001.

Mathimani T, Sekar M, Shanmugam S, Sabir JSM, Chi NTL, Pugazhendhi A. 2021. 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. *Science of the Total Environment*. vol 771(144700). doi: https://doi.org/10.1016/j.scitotenv.2020.144700.

Mühlsteinová R, Hauer T, De Ley P, Pietrasiak N. 2018. Seeking the true *Oscillatoria*: A quest for a reliable phylogenetic and taxonomic reference point. *Preslia*. vol 90(2): 151–169. doi: https://doi.org/10.23855/preslia.2018.151.

Natunen K. 2020. Monitoring microalgal neutral lipid accumulation with Nile Red. In: *Methods in Molecular Biology*. vol 1980: 55–61. Humana Press Inc. doi: https://doi.org/10.1007/7651_2017_102.

Neto JM, Komesu A, Martins LHdaS, Gonçalves VOO, Oliveira JARde, Rai M. 2019. Third generation biofuels: An overview. In: *Sustainable Bioenergy: Advances and Impacts*: 283–298. Elsevier. doi: https://doi.org/10.1016/B978-0-12-817654-2.00010-1.

Nicodemou A, Konstantinou D, Koutinas M. 2024. Enhanced biomass and lipid production from olive processing wastewater using *Scenedesmus obliquus* in a two-stage cultivation strategy under salt stress. *Biochemical Engineering Journal*. vol 205(109290). doi: https://doi.org/10.1016/j.bej.2024.109290.

Noerdjito DR, Windarsih A, Purbani DC, Purnaningsih I, Suratno. 2024. Untargeted metabolomics profiling of tropical marine microalgae in batch culture system with different mobile phase using liquid chromatography-Orbitrap high-resolution mass spectrometry combined with chemometrics. *Regional Studies in Marine Science*. vol 79(103834). doi: https://doi.org/10.1016/j.rsma.2024.103834.

Nunes NSP, Ansilago M, De Oliveira NN, Leite RSR, Da Paz MF, Fonseca GG. 2021. Biofuel production. In: *Microalgae: Cultivation, Recovery of Compounds and Applications*: 145–171. Elsevier. doi: https://doi.org/10.1016/B978-0-12-821218-9.00006-2.

Nur MMA. 2014. Potency of microalgae as source of functional food in Indonesia (overview). *Eksergi*. vol 11(2): 1-6. doi: https://doi.org/10.31315/e.v11i2.363.

Ogbonna KE, Ogbonna JC, Njoku OU, Yamada K, Suzuki I. 2024. Effect of organic carbon sources on growth, lipid production and fatty acid profile in mixotrophic culture of *Scenedesmus dimorphus* (Turpin) Kützing. *The Microbe*. vol 3(100064): 1-11. doi: https://doi.org/10.1016/j.microb.2024.100064.

Pachiappan P, Prasath BB, Perumal S, Ananth S, Devi AS, Kumar SD, Jeyanthi S. 2015. Isolation and culture of microalgae. In: *Advances in Marine and Brackishwater Aquaculture*: 1–15. Springer India. doi: https://doi.org/10.1007/978-81-322-2271-2_1.

Pan YY, Wang ST, Chuang LTe, Chang YW, Chen CNN. 2011. Isolation of thermo-tolerant and high lipid content green microalgae: Oil accumulation is predominantly controlled by photosystem efficiency during stress treatments in *Desmodesmus*. *Bioresource Technology*. vol 102(22): 10510–10517. doi: https://doi.org/10.1016/j.biortech.2011.08.091.

Pane EP, Risjani Y, Yunianta, Pane J, Fitrianesia F, Nugraha R, Rahmawati K. 2023. Description of some microalgae isolates captured from Sumatra Island, Indonesia. *IOP Conference Series: Earth and Environmental Science*. vol 1191(012014): 1-9. doi: https://doi.org/10.1088/1755-1315/1191/1/012014.

Priyanka P, Kinsella GK, Henehan GT, Ryan B. 2020. Nile Red assay development for the estimation of neutral lipids in *Chlorella emersonii* and *Pseudokirchneriella subcapitata*. *Eurobiotech Journal*. vol 4(4): 216–222. doi: https://doi.org/10.2478/ebtj-2020-0025.

Rahman DY, Praharyawan S, Fadiyah F, Raharjo S, Susilaningsih D. 2019. Morphology and molecular characterization of newly isolated microalgae strain *Chlorela volutis* LIPI13-WKT066 from Wakatobi Islands and its potential use. *Annales Bogorienses*. vol 23(1): 13–19. doi: https://doi.org/10.14203/ann.bogor.2019.v22.n2.13-19.

Rani VU, Perumal UE, Palanivel S. 2016. Morphology and taxonomy of *Oscillatoria princeps* Vaucher ex Gomont (Oscillatoriales, Oscillatoriaceae). *Indian Journal of Education and Information Management*. vol 5(1): 1-5.

Rinanti A, Fachrul MF, Hadisoebroto R, Desty S, Rahmadhania R, Widyaningrum DA, Saad NA. 2021. Heavy metal pollutant sorption in aquatic environment by microalgae consortium. *Indonesian Journal of Urban and Environmental Technology*. vol 5(1): 51–71. doi: https://doi.org/10.25105/urbanenvirotech.v5i1.10747.

Rod-In W, Monmai C, Shin IS, You SG, Park WJ. 2020. Neutral lipids, glycolipids, and phospholipids isolated from sandfish (*Arctoscopus japonicus*) eggs exhibit anti-inflammatory activity in LPS-stimulated RAW264.7 cells through NF-κB and MAPKs pathways. *Marine Drugs*. vol 18(480): 1-14. doi: https://doi.org/10.3390/md18090480.

Santosa AD. 2010. Lipid producing microalgae from several ecosystems in West and Central Jawa, Indonesia. *Biotropia*. vol 17(2): 98-104. doi: https://doi.org/10.11598/btb.2010.17.2.79.

Saputro TB, Purwani KI, Ermavitalini D, Saifulloh AF. 2019. Isolation of high lipid content microalgae from Wonorejo River, Surabaya, Indonesia and its identification using rbcL marker gene. *Biodiversitas*. vol 20(5): 1380–1388. doi: https://doi.org/10.13057/biodiv/d200530.

Sharma J, Kumar SS, Bishnoi NR, Pugazhendhi A. 2019. Screening and enrichment of high lipid producing microalgal consortia. *Journal of Photochemistry and Photobiology B: Biology*. vol 192: 8–12. doi: https://doi.org/10.1016/j.jphotobiol.2019.01.002.

Silambarasan S, Logeswari P, Sivaramakrishnan R, Incharoensakdi A, Kamaraj B, Cornejo P. 2023. *Scenedesmus* sp. strain SD07 cultivation in municipal wastewater for pollutant removal and production of lipid and exopolysaccharides. *Environmental Research*. vol 218(115051). doi: https://doi.org/10.1016/j.envres.2022.115051.

Singh N, Saluja RK, Rao HJ, Kaushal R, Gahlot NK, Suyambulingam I, Sanjay MR, Divakaran D, Siengchin S. 2024. Progress and facts on biodiesel generations, production methods, influencing factors, and reactors: A comprehensive review from 2000 to 2023. *Energy Conversion and Management*. vol 302. Elsevier Ltd. doi: https://doi.org/10.1016/j.enconman.2024.118157.

Trivedi J, Atray N, Agrawal D, Ray A. 2022. Enhanced lipid production in *Scenedesmus obliquus* via nitrogen starvation in a two-stage cultivation process and evaluation for biodiesel production. *Fuel*. vol 316(123418). doi: https://doi.org/10.1016/j.fuel.2022.123418.

Vignesh P, Kumar ARP, Ganesh NS, Jayaseelan V, Sudhakar K. 2021. Biodiesel and green diesel generation: An overview. *Oil and Gas Science and Technology*. vol 76(6): 1-15. doi: https://doi.org/10.2516/ogst/2020088.

Wahyuningtyas ASH, Abidin Z, Putri WDR, Maligan JM, Berlian GO, Ningrum PFW. 2024. Consumer's willingness to try new microalgae-based food in Indonesia. *Journal of Agriculture and Food Research*. vol 18(101367): 1-13. doi: https://doi.org/10.1016/j.jafr.2024.101367.

Yudana IGPGR, Suprakto B, Undu MC, Insani L, Fiqriyah A, Charunisa A, Harijono T, Kiswanto A, Aras AC, Ayu D, Zahid A, Wahyu, Fauziah A. 2021. Distribution of microalgae as bioindicator for *Sardinella lemuru* in Bali Strait. *IOP Conference Series: Earth and Environmental Science*. vol 860(012009): 1-11. doi: https://doi.org/10.1088/1755-1315/860/1/012009.

Zakiyah U, Mulyanto, Suwanti LT, Koerniawan MD, Suyono EA, Budiman A, Siregar UJ. 2020. Diversity and distribution of microalgae in coastal areas of East Java, Indonesia. *Biodiversitas*. vol 21(3): 1149–1159. doi: https://doi.org/10.13057/biodiv/d210340.

Zhang S, Zhang L, Xu G, Li F, Li X. 2022. A review on biodiesel production from microalgae: Influencing parameters and recent advanced technologies. *Frontiers in Microbiology*. vol 13(970028): 1-20. doi: https://doi.org/10.3389/fmicb.2022.970028.

Zhang SS, Liu H, Fan JF, Yu H. 2015. Cultivation of *Scenedesmus dimorphus* with domestic secondary effluent and energy evaluation for biodiesel production. *Environmental Technology (United Kingdom)*. vol 36(7): 929–936. doi: https://doi.org/10.1080/09593330.2014.966769.