Malaysian Journal of Chemistry, 2026, Vol. 28(1), 270-275
(Special Issue: *3rd International Conference on Industry-Academia Initiatives in Biotechnology & Chemistry, iCIABC2025*)

Cultivation of *Nannochloropsis oculata* in Petroleum Wastewater under Different Monochromatic Light-emitting Diode (LED) Light for Biodiesel Production

Primilla Paramasivam[1], Karthiani Kanagesan[1], Natanamurugaraj Govindan[2] and Gaanty Pragas Maniam[1]*

[1]Algae Culture Collection Center & Laboratory, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Persiaran Tun Khalil Yaakob, 26300 Kuantan, Pahang, Malaysia

[2]Department of Botany and Microbiology, Hemvati Nandan Bahuguna Garhwal University, Chauras Campus Srinagar Garhwal, Uttarakhand 249161, India

*Corresponding author (e-mail: gaanty@umpsa.edu.my)

Microalgae have the potential as a source of biodiesel due to their high lipid productivity and ability to grow in variety of environments, including wastewater. Therefore, in this study isolated species *Nannochloropsis oculata* are cultured in 10% (v/v) petroleum wastewater for 18:06 hours light/dark (L/D) under various monochromatic light-emitting diode (LED) lights: blue (λ = 465 nm), red (λ = 660 nm), green (λ = 520 nm), and fluorescent (full-spectrum) as a control he studies indicated that *N. oculata* grown under blue LED illumination had a high fat percentage of 63.3 % and a total dry weight of 0.988 ± 0.04g L^{-1}. The potential *N. oculata* which cultivated under blue light generated the greatest amount of fatty acid methyl ester (FAME) at 81.1 %. It was also able to remove up to 94.2% of BOD, 89.2% of COD, and 94.8% of wastewater pollutants.

Keywords: *Nannochloropsis* sp., petroleum wastewater, light wavelength, biodiesel, light-emitting diode

Received: January 2025; Accepted: December 2025

The global demand for sustainable and renewable energy sources has accelerated research into alternative fuels, with biodiesel from microalgae emerging as one of the most promising candidates. The reliance on fossil fuels has led to severe environmental concerns, including greenhouse gas emissions, climate change, and depletion of non-renewable resources. Microalgae, particularly *Nannochloropsis* species, have drawn attention due to their rapid growth, high lipid productivity, and ability to thrive in non-arable environments [1-2].

Microalgae offer a dual benefit in the context of biodiesel production. Beyond lipid accumulation, they also play a crucial role in environmental management by assimilating nutrients and pollutants from wastewater. This co-benefit is critical given the high costs associated with conventional wastewater treatment technologies. Blue (450 nm) and red (620 nm) LEDs are frequently reported to enhance microalgae growth due to their strong overlap with chlorophyll-a absorption peaks, thereby maximizing photosynthetic efficiency [3-4]. However, most previous studies were conducted in standard synthetic media such as BG-11, rather than in nutrient-rich industrial wastewater. As a result, the combined effect of different LED wavelengths and petroleum wastewater on *Nannochloropsis oculata* growth and lipid biosynthesis remains insufficiently understood, representing a critical knowledge gap. Addressing this gap is essential to determine whether low-cost wastewater resources, when paired with optimized light conditions, can effectively support sustainable biodiesel production.

LEDs not only enhanced biomass productivity but also increased eicosapentaenoic acid (EPA) yields, providing evidence of wavelength-specific metabolic optimization. Similarly, Singh and Gu (2010) highlighted that blue light conditions significantly boosted lipid synthesis compared to green wavelengths, aligning with chlorophyll-a absorption peaks [5].

Wastewater as a cultivation medium has been widely studied for reducing production costs. A 2025 study utilizing palm oil mill effluent (POME) under LED illumination reported lipid yields exceeding 55%, with simultaneous removal of more than 85% of COD and BOD (Environmental Progress & Sustainable Energy, 2025). Comparable results were reported by Guzman et al. (2025), where *Nannochloropsis oculata* achieved up to 96% COD removal when combined with dissolved air flotation (DAF) pre-treatment [6]. These findings demonstrate the potential of integrating microalgal cultivation into wastewater management frameworks.

†Paper presented at the 3rd International Conference on Industry-Academia Initiatives in Biotechnology & Chemistry

Despite these advancements, relatively few studies have investigated the combined effect of petroleum wastewater and monochromatic LED lighting on *Nannochloropsis* cultivation. Petroleum wastewater presents unique challenges due to its complex mixture of hydrocarbons and heavy metals. However, the probability of success is supported by prior evidence showing pollutant removal efficiencies exceeding 90% under similar microalgal systems [2,7]. Given that *Nannochloropsis* under blue LED has demonstrated lipid content above 60% [3], the likelihood of achieving biodiesel yields surpassing 80% FAME conversion is high, making this study a valuable contribution to the field.

Therefore, this research aims to explore the growth performance, lipid productivity, and wastewater remediation potential of *Nannochloropsis oculata* cultivated under blue, red, and green LED wavelengths in petroleum wastewater. By comparing expected outcomes with established studies, this work intends to provide a novel perspective on integrating renewable energy production with industrial wastewater management.

EXPERIMENTAL

Sample Collection, Isolation, and Identification of Microalgae

Water samples were collected from Pantai Teluk Cempedak, Kuantan, Pahang, Malaysia, using a plankton net. The collected samples were centrifuged at 3000 rpm for 10 minutes to concentrate the algal cells and remove suspended debris. The pellet was resuspended in sterile BG-11 medium and incubated on a laboratory rack under continuous fluorescent illumination (1500 ± 100 lux) with aeration supplied through filtered air. The cultures were maintained until visible sign of algal growth was observed. The growing culture was serially diluted using sterile distilled water. These diluted samples were plated onto a solidified BG-11 agar medium and fourteen days of incubation. The morphological features of the isolated colonies were examined using a field emission scanning electron microscope and a fluorescence microscope. After that, the culture was increased to 500, 1000, and 2000 mL, respectively.

Petroleum Wastewater Medium Preparation

Petroleum wastewater was collected from the Petronas Refinery, Melaka and stored in plastic container at 4 °C to avoid contamination and biodegradation. The suspended solids of the wastewater were removed by filtering them using a filter cloth and centrifuged at 8000 rpm for 10 min. Eliminating suspended particles improves light penetration which supports microalgae's photosynthetic activity. The supernatant that acquired which contained nutrients from microalgae culture. Later, the wastewater was diluted to 10% (v/v) using distilled water, which is 100 mL of petroleum wastewater was made up to 1 L with distilled water. Wastewater dilution enhances light penetration into the medium. After adjusting the diluted medium's pH to between 7.0 – 7.5, it was autoclaved for 20 minutes at 121 °C to eliminate any native pollutants. Prior to use, the petroleum wastewater medium was re-filtered through Grade 1 Whatman Filter papers with the diameter of 150mm and the pH was re-adjusted again to the range of 7.0 – 7.5 after autoclaving.

Cultivation of Nannochloropsis Oculate under Different Wavelengths of LED Light

The 10% POME and 18:06 h L/D light regime were chosen as constant factors in this study to better examine the effects of monochromatic LED light wavelength optimization. For 18:06 hours L/D, *Nannochloropsis oculata* was grown in 2 L Erlenmeyer flasks with a 10% POME solution. The *N. oculata* culture was maintained in three separate enclosed boxes with three distinct monochromatic LED lights - blue (465 nm), red (660 nm), green (520 nm), and fluorescent as control in a full-spectrum, during the 18-hour light phase. For the next six hours of the dark phase, all of the cultures were kept in enclosed, dark boxes and covered with aluminum foil. The cultures were continuously aerated with sterile-filtered air and maintained at 25 ± 2 °C.

Growth Analysis of *Nannochloropsis oculate*

Dry weight (DW) and optical density (OD) were used to analyze the growth of *N. oculata*. The optical density of a culture of *Nannochloropsis oculata* culture was measured at 680nm using a spectrophotometer to monitor growth. gravimetric analysis of the dry cell weight was performed every three days. Regular centrifugation of microalgae culture aliquots was carried out for 10 minutes at 6,000 rpm. Following collection, the pellet was dried and weighed before being put in a glass petri dish. Before the samples were weighed, they were dried. The dry weights were specified as g L^{-1}.

Extraction of Lipid

Crude oil was extracted from dried biomass using the technique developed by [8]. A centrifuge tube containing 1.25 grams of dried biomass sample was used (ISOLAB, Germany). After that, 12.5 mL of a 2:1 v/v chloroform: methanol combination was added to the biomass, and it was allowed to soak in the organic solvents for four hours at room temperature while being continuously shaken at 150 rpm using an IKA® KS 260 basic rotary shaker. The lipid with solvent was then moved into a separating funnel after the mixture had been centrifuged for five minutes at 4000 rpm. After adding 5 mL of distilled water to the mixture the oil was separated from the solvent.

A rotary flask was used to collect the oil that contained PUFAs that were discovered in the organic phase (bottom layers). A rotary evaporator was used to aspirate the chloroform layer from the oil and the separated biomass.

Transesterification

10 mg of *Nannochloropsis oculata* lipid, 200 µL of 2 M methanolic KOH as the catalyst, and 2 mL of hexane were combined to transesterify the lipid into FAME. The liquid was vigorously stirred for five minutes with a vortex (8031102000 MX-S, DLAB, USA). TLC analysis was conducted using silica gel 60 F254 aluminium plates (Merck, Germany). The mobile phase consisted of hexane: diethyl ether: acetic acid (70:30:1 v/v/v). Samples using a micro-capillary tube and developed until the solvent migrated 8cm from the origin. Plates were visualized under UV light (254 nm).

RESULTS AND DISCUSSION

Isolation and Identification of *Nannochloropsis oculata*

Six species of microalgae, (*Chlorella* sp., *Amphora* sp., *Gyrosigma* sp., *Tetraselmis* sp., *Spirulina* sp., and *N. oculata*) were identified and located from the fresh water collected in Teluk Cempedak, Kuantan, Pahang, Malaysia.

Compared to other microalgae, *N. oculata* shown exceptional development and visibility throughout the early stages of cultivation. In Figure 1, shows the outcome flururesence microscope image of *N.oculata*.

Cultivation of *Nannochloropsis Oculata* under Different Wavelengths of LED Light

The growth profile of *N. oculata* cultured under green, red, blue, and fluorescent LED lights is shown in Figure 2. Across all treatments, cultures exhibited a slow increase in biomass during the first two days, followed by a steady exponential phase until Day 10. This trend indicates successful acclimatization to the medium and efficient photosynthetic activity during the early phase of cultivation [9]. After Day 10, growth of all cultures began to slow due to nutrient depletion and self-shading, marking the onset of the stationary phase.

Among all wavelengths tested, blue LED lighting resulted in the highest cell density throughout the cultivation period. On day 14, the blue-LED treatment reached an OD680 of 1.567, which was substantially higher than cultures exposed to red and fluorescent LEDs, and more than double the biomass recorded under green LED illumination. The darker green medium observed under blue and red LEDs further suggests a higher chlorophyll concentration and cellular density in these treatments.

The superior performance under blue wavelengths is consistent with previous findings that short-wavelength, high-energy photons penetrate cultures more effectively and enhance photochemical efficiency [10]. Blue light closely matches the absorption maxima of chlorophyll a (around 465 nm), enabling stronger photosystem II excitation and improved electron transport [11]. In contrast, green light is poorly absorbed and therefore supports lower biomass accumulation.

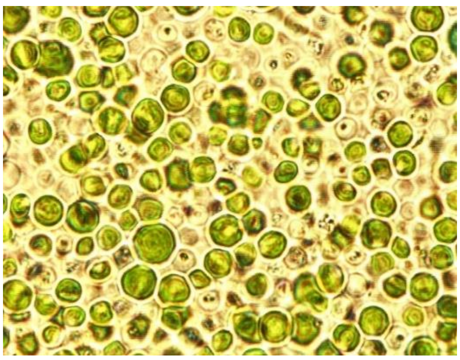

Figure 1. Identified strain of *Nannochloropsis* oculata strain,

Several studies have demonstrated that blue-light exposure increases CO_2 fixation rates by promoting the activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and carbonic anhydrase [9]. This aligns with the present observation that blue LED cultures maintained prolonged exponential growth, leading to significantly greater biomass productivity.

In addition to growth performance, blue-light treatment also improved lipid accumulation. *N. oculata* grown under blue LED achieved 0.415 ± 0.003 g L^{-1} of total lipid, corresponding to 52.7% of dry biomass, outperforming red, green, and fluorescent light treatments. This behaviour is well-supported in microalgal research, as blue wavelengths can upregulate key genes in fatty-acid biosynthesis pathways and stimulate triacylglycerol (TAG) storage [11].

Overall, the current findings confirm that blue LED light is the most effective wavelength for enhancing both cellular productivity and storage lipid content in *N. oculata*, making it the most suitable wavelength for future optimization in biodiesel applications.

Furthermore, the petroleum wastewater medium provided a more favorable carbon source for *Nannochloropsis oculata* growth compared to the conventional BG-11 medium, owing to its high organic content and nutrient load [12]. Photosynthetic CO_2 fixation by the microalgae shifted the equilibrium, compensating for the carbon demand during biomass accumulation. The efficient photosynthetic metabolism of *N. oculata* enhanced O_2 evolution, which subsequently reacted with free hydrogen ions to form hydroxyl radicals, contributing to stable pH conditions during cultivation. To minimize drastic fluctuations, a continuous supply of CO_2 was maintained throughout the process, which has been shown to significantly improve microalgal growth in wastewater-based media.

In line with previous findings that wastewater-derived nutrients can maintain or even increase lipid productivity, petroleum wastewater also had enough nitrogen and phosphate to support algal growth, successfully replacing synthetic nutrients from BG-11 [13].

Lipid Extraction of Microalgae

Lipid content and biomass concentration were evaluated to determine how petroleum wastewater supported lipid productivity of *Nannochloropsis oculata* under different LED wavelengths. Cultures grown under blue LED illumination in petroleum effluent showed continuous biomass growth throughout the cultivation period, reaching the stationary phase around day 10 (Figure 2). At this time, the recovered dry biomass had a lipid content of 63.3% (≈0.63 g/L), which was markedly higher than the 42% (≈0.42 g/L) produced under BG-11 medium using the same light condition. This indicates that *N. oculata* was able to effectively utilize the nutrients present in petroleum effluent during the exponential phase, thereby enhancing cell division and improving lipid accumulation under blue light. This observation is consistent with previous studies reporting that shorter wavelengths, especially blue light (~465 nm), stimulate photosynthetic activity, carbon fixation, and lipid biosynthesis in *Nannochloropsis sp.* [1-2, 14].

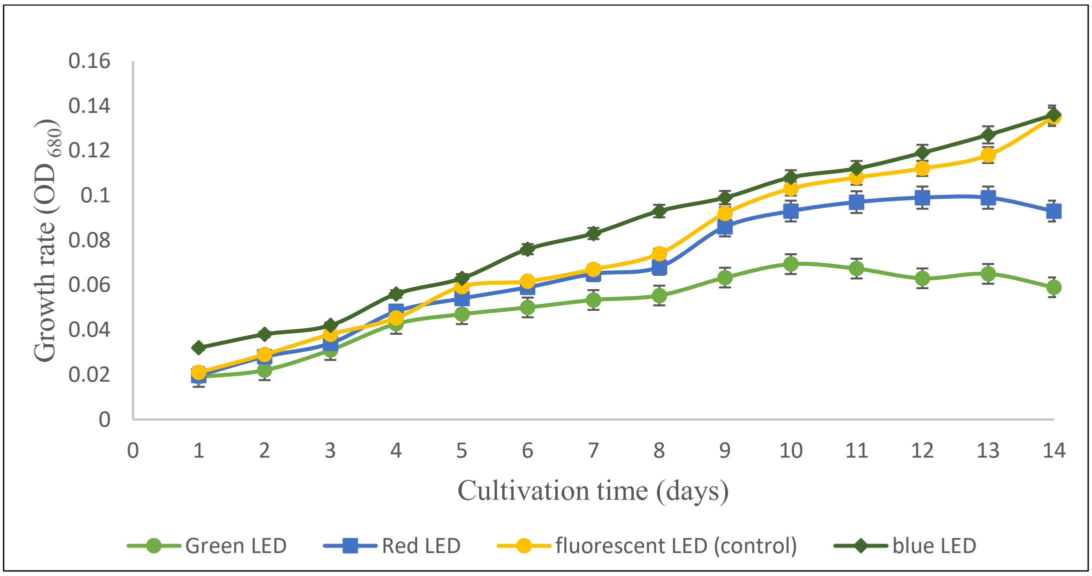

Figure 2. Growth rate of *Nannochloropsis oculata* in various LED wavelength.

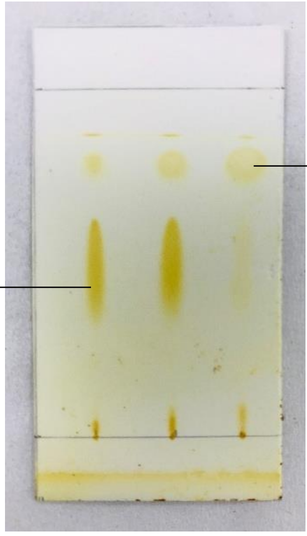

Figure 3. TLC profile of *Nannochloropsis oculata* lipids.

These results are supported by recent research highlighting the potential of wastewater as an inexpensive, nutrient-rich substrate for lipid synthesis. Tamil Selvan et al. (2024) demonstrated that *Nannochloropsis salina* cultivated in cassava effluent achieved >60% lipid content, while Kiani et al. (2024) reported that *Nannochloropsis oceanica* grown in whey permeate not only increased essential fatty acid content but also accumulated high lipid yields, supporting wastewater valorization for biodiesel production [15-16]. Furthermore, Zaimes et al. (2025) showed that *Nannochloropsis gaditana* grown in petroleum-refinery wastewater achieved ~37% lipid yield while simultaneously lowering COD and BOD levels, confirming the dual benefit of nutrient removal and biofuel feedstock generation [17]. Collectively, these findings demonstrate that petroleum wastewater is a reliable and sustainable substitute for synthetic media such as BG-11, with the added advantage of enhanced lipid productivity under blue LED illumination.

Thin Layer Chromatography (TLC) Analysis

The lipid profile of *Nannochloropsis oculata* cultivated in 10% petroleum wastewater under LED illumination was analyzed using Thin Layer Chromatography (TLC). As shown in Figure 3, distinct lipid fractions were clearly visible. The major bands observed corresponded to triacylglycerols (TAGs), which are the primary feedstock for biodiesel production, as well as bands associated with free fatty acids (FFAs) and phospholipids. The Rf values obtained for the prominent bands ranged from 0.30 to 0.85, consistent with previously reported lipid fractions from *Nannochloropsis* cultures [14-15].

The intensity of the TAG band was strongest in samples cultivated under blue LED illumination, aligning with the higher lipid yield (63.3%) recorded in this treatment. The presence of a clear and concentrated TAG band suggests a high potential for biodiesel production, as TAGs are directly converted into fatty acid methyl esters (FAMEs) during transesterification. Compared to cultures under other light treatments, the TLC results indicate that blue LED provided optimal conditions for lipid biosynthesis, corroborating findings from recent studies that blue light enhances lipid accumulation in microalgae [16]. Although further analysis such as GC-MS could provide detailed fatty acid composition, TLC has proven sufficient for verifying the presence of biodiesel-relevant lipid classes, confirming the suitability of this cultivation strategy for biodiesel production.

CONCLUSION

This study demonstrated that *Nannochloropsis oculata* cultivated in 10% petroleum wastewater showed higher biomass accumulation under blue LED light compared to other light wavelengths. The enhanced growth achieved using wastewater-derived medium indicates its potential as an economical alternative to BG-11 for algal cultivation. Future studies focusing on nutrient removal efficiency and lipid upgrading will be necessary to fully validate wastewater valorisation benefits.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA) for financial support through research grant RDU233307 (International Publication Grant).

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