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Impact of Various Visible Spectra on Attached Microalgal Growth on Palm Decanter Cake in Triggering Protein, Carbohydrate, and Lipid to Biodiesel Production

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Abstract: Attached microalgal growth of *Chlorella vulgaris* on palm decanter cake (PDC) under irradiation with various visible monochromatic and polychromatic spectra to produce biodiesel was studied in this work. The results demonstrated that the white spectrum cultivation exhibited the highest microalgal density of 1.13 g/g along with 1.213 g/L day of microalgal productivity. Correspondingly, the biodiesel obtained was comprised mainly of C16 and C18 fatty acids, possessing a high cetane number and oxidation stability from the high saturated fatty acid content (70.38%), which was appealing in terms of most biodiesel production requirements. Nevertheless, the highest lipid content (14.341%) and lipid productivity (93.428 mg/L per day) were discovered with green spectrum cultivation. Blue and white spectra led to similar protein contents (34%) as well as carbohydrate contents (61%), corroborating PDC as a feasible carbon and nutrient source for growing microalgae. Lastly, the energy feasibilities of growing the attached microalgae under visible spectra were investigated, with the highest net energy ratio (NER) of 0.302 found for the yellow spectrum. This value outweighed that in many other works which have used suspended growth systems to produce microalgal fuel feedstock. The microalgal growth attached to PDC is deemed to be a suitable alternative cultivation mode for producing sustainable microalgal feedstock for the biofuel industry.

Keywords: microalgae; visible spectrum; biodiesel; attached growth; palm decanter cake; net energy ratio

1. Introduction

There has been an increase in population and urbanization over the last few decades as a result of global industrialization. Consequently, the need for fossil fuels has increased

significantly. This has inevitably depleted the stores of fossil fuels since they are non-renewable resources. According to BP's statistical analysis report, fossil fuels will be completely exhausted within the next 50 years if they are exploited continuously at the current rate [1]. Besides, fossil fuels also create environmental disadvantages, mainly contributing to the greenhouse effect due to the emission of carbon dioxide (CO_2) from fossil fuel combustion. Thus, microalga have been found to be a sustainable feedstock for producing biofuels with astounding benefits [2,3]. Microalgae have a high growth rate and lipid content, and do not compete for agricultural land. Notably, they could sequester CO_2 for assuaging global warming. Furthermore, to date, biodiesel from microalgal cultivation has been projected to be the front runner for replacing diesel fuel because of the high cetane number and low sulfur content [1].

Generally, there are four types of microalgal cultivation modes: photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic, with the high biomass productivity usually being reported under mixotrophic cultivation [4]. Moreover, compared with the suspended cultivation system, numerous researchers have indicated that the attached cultivation system exhibits better harvesting performance and higher biomass yield as well as lower water and energy consumption [5]. Hence, attached microalgal cultivation has become the predominant choice for growing microalgae. In the attached cultivation system, the microalgae are grown on a solid support to form a biofilm. A number of support materials had been investigated, such as filter paper, glass fiber, fabric–hydrogel composites, polytetrafluoroethylene, etc. Nonetheless, these substrates are not ideal for long-term cultivation, as their performances would deteriorate after multiple re-culturing [6]. In fact, they lack the essential amounts of nutrients for microalgal growth, which incurs the cost of supplementary nutrients in the culture medium. In spite of that, attached microalgal cultivation has been widely explored. With the aim of reducing operational costs, agricultural wastes such as palm decanter cake (PDC) have been proposed for use as support media for attached microalgal cultivation, which was initially postulated to proffer both nutrients and a platform for biofilm formation. Interestingly, PDC has also been reported as a source of nutrients for plantations [7]. Furthermore, PDC can be easily collected from palm oil milling plants as a waste by-product of oil palm plantations that is prevalent in Southeast Asia.

The effect of the visible spectrum stands among the least studied factors for stimulating microalgal growth. Recent breakthroughs in microalgal cultivation have revealed the plausible potential influence of monochromatic and polychromatic LED wavelengths on enhanced microalgal growth [8]. This has also been supported by various studies on which pigments produced by the microalgae absorb light energy. When a light quantum is absorbed, these pigment molecules are stimulated and transformed to a state of high energy. When they return to their original condition, they release energy, which drives photochemical reactions. The energy hub of microalgae, known as the photosystem, contains a distinctive set of light-harvesting pigments that provide a unique absorption spectrum. Therefore, different spectral compositions of light are known to be effectively captured by distinct chlorophyll pigments, resulting in enhanced microalgal growth [9].

Therefore, this study highlighted the efficacy of attached microalga (i.e., *Chlorella vulgaris*) on PDC under irradiation by polychromatic and various monochromatic visible spectra to enhance microalgal growth and lipid production. In this regard, a novel use of solid nutrients was adopted to culture the microalgal cells on the PDC's surface. Additionally, the quality and composition of fatty acid methyl esters (FAME) in the biodiesel was examined, as well as an energy feasibility assessment to confirm the viability of the microalgal biodiesel production process.

2. Materials and Methods

2.1. Cultivation of Microalgal Stock

A strain of bacteria-free *Chlorella vulgaris* sp. USMAC 24 was used as the inoculum. The microalgal culture was acquired from the Centre for Biofuel and Biochemical Re-

search, Universiti Teknologi PETRONAS, and was carried out in synthetic Bold's Basal medium (BBM) until the required amount for carrying out the batch experiments had been obtained. The media had the following chemical compositions: (1) 10 mL/L of NaCl (2.5 g/L), $MgSO_4 \bullet 7H_2O$ (7.5 g/L), $CaCl_2 \bullet 2H_2O$ (2.5 g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), and $NaNO_3$ (25 g/L) and (2) 1 mL/L of $Co(NO_3)_2 \bullet 6H_2O$ (0.49 g/L), H_3BO_3 (11.4 g/L), $CuSO_4 \bullet 5H_2O$ (1.57 g/L), $ZnSO_4 \bullet 7H_2O$ (8.82 g/L), $FeSO_4 \bullet 7H_2O$ (4.98 g/L), $MnCl_2 \bullet 4H_2O$ (1.44 g/L), MoO_3 (0.71 g/L), anhydrous EDTA (50 g/L), KOH (31 g/L), and H_2SO_4 (1 mL/L) [3]. A microalgal seed of 500 mL was first introduced in a 5 L bottle containing 4.5 L of the medium. Compressed air at the flow rate of 6.5 L/min was aerated into the cultured medium at room temperature (25 °C ± 1 °C). Simultaneously, the culture was exposed to continuous illumination under a cool-white, fluorescent light at an intensity of 60–70 μmol/m^2s. The pH of the medium was kept constant at 3.0 ± 0.1 throughout the cultivation period. The cultivation process was ended once the microalgal growth reached a stationary growth phase prior to experimental use [10].

2.2. Characterization of the Palm Decanter Cake

The palm decanter cake (PDC) was collected from the Nutrition Technologies Sdn Bhd. The PDC was then stored at temperatures below 4 °C in a chiller before being subjected to analysis. The CHNS analyzer was used to determine the elemental content (C, H, N) of the PDC. Oxygen gas was required to oxidize the subjected elements into their oxide molecules at 1000 °C. An inert carrier gas, namely helium, was used to sweep the combusted products and pass them over to heated high-purity copper at 600 °C, in which the copper was responsible for reduction of the molecules [10]. Determination of the moisture content and dry matter of the PDC were carried out using a standard oven-drying method: IS 2720-2 (1973).

2.3. Experimental Setup for Attached Microalgal Growth

Attached microalgal cultivation was carried out in 1 L Erlenmeyer flasks that served as the photobioreactors. Initially, each 1 L Erlenmeyer flask contained 900 mL of tap water to which 100 mL of the microalgal stock culture was added, with the pH being kept constant at 3.0 ± 0.1 throughout the cultivation period. Next, PDC at a concentration of 10 g/L was added into each cultivation flask to act as a nutritional support substratum. All setups were aerated with compressed air at a flow rate of 1.3 L/min at room temperature (25 °C ± 1 °C). Different colors of lights were used to separately illuminate every photobioreactor containing the attached microalgal growth on PDC. In this regard, five lightbulbs with different irradiant spectrum colors, namely, red, blue, white, yellow, and green, were used at an intensity of 100 μmol/m^2s each. The cultivation period was scheduled for 9 days of exposure to each irradiant spectrum before measuring the microalgal biomass and lipid production. The flasks were then sealed with aluminum foil to prevent air contamination. Each setup was covered with a black box to minimize random light radiation from the surroundings. To ensure the consistency of results, each cultivation flask was at least duplicated [10,11].

2.4. Analytical Methods

2.4.1. Attached Microalgal Biomass Productivity

Gravitational sedimentation was adopted to harvest the mature attached microalgae from the PDC. The aeration was stopped and the attached microalgae with residual PDC was allowed to settle at the bottom of the culture medium. The supernatant was then decanted carefully without agitating the attached microalgae on the PDC layer. The remaining biomass was then subjected to centrifugation at 6000 rpm for 6 min for further dewatering. The PDC with attached microalgal biomass was subsequently dried in an oven at 60 °C until a constant weight was achieved (W_1). After that, the residual PDC was separated from the microalgal biomass by introducing mixed solvents at the ratio of 2:1 of methanol and chloroform, respectively. The mixture in the solution was gently stirred until a suspension of the residual PDC appeared, which was isolated physically. The solution

was then rested, and the separated residual PDC was dried at 60 °C before being weighed as W_2. Thereafter, the attached microalgal biomass productivity was calculated by using Equation (1) [12]:

$$\text{Attached microalgal biomass productivity} \left(\frac{\text{g}}{\text{L day}}\right) = \frac{\text{Dry attached microalgae (g)}}{\text{Cultivation volume (L)} \times \text{Cultivation period (day)}} \quad (1)$$

where dry attached microalgae were calculated by subtracting W_1 from W_2 [10].

2.4.2. Attached Microalgal Biochemical Productivity

The microalgal residue in the chloroform and methanol mixture was transferred into a capped sampling bottle and placed on an orbital shaker for 24 h at 250 rpm. Thereafter, the solvent mixture was filtered by using Whatman Grade 1 filter paper to retain the solid microalgal biomass. The solvent filtrate was evaporated by purging with dry inert gas to obtain the extracted microalgal lipids. Finally, the lipid content Equation (2) and lipid productivity Equation (3) were calculated from weighing the residue in the glass vial [10].

$$\text{Lipid content (\%)} = \frac{\text{Lipid yield (g)}}{\text{Dry attached microalgae (g)}} \times 100\% \quad (2)$$

$$\text{Lipid productivity} \left(\frac{\text{mg}}{\text{L day}}\right) = \frac{\text{Lipid yield (mg)}}{\text{Cultivation volume (L)} \times \text{Cultivation period (day)}} \quad (3)$$

Subsequently, the protein content was determined on the basis of the nitrogen content in the microalgae by Equation (4). The carbohydrate content was calculated by subtracting the lipid and protein contents from 100%.

$$\text{Protein content (\%)} = \frac{\text{Nitrogen content in attached microalgae(g)} \times 5.3}{\text{Dry attached microalgae (g)}} \times 100\% \quad (4)$$

The multiplication factor of 5.3 was proposed to correlate the nitrogen and protein content in palm decanter cake [13]. The nitrogen content in the attached microalgae was verified by analyzing the nitrogen content of the microalgal stock culture in a CHNS analyzer and multiplied by the weight of the microalgae after lipid extraction (Equation (5)) [11].

$$\text{Microalgae weight after lipid extraction (g)} = [100\% - \text{Lipid content (\%)}] \times \text{Dry attached microalgae (g)} \quad (5)$$

2.4.3. Profile of Fatty Acid Methyl Esters

Sample mixing was enhanced by introducing 1 mL of tetrahydrofuran to the extracted lipid. After that, methanol and lipid at a 15:1 ratio in 3 wt.% of concentrated sulfuric acid catalyst were mixed thoroughly before initiating the transesterification process in an incubator shaker for 3 h at 60 °C. After that, 2 mL of methanol, 10 mL of hexane, 4 mL of 10% sodium chloride, and 4 mL of distilled water were added and mixed thoroughly. The solvent mixture was later transferred into a centrifugation tube and centrifuged at 5000 rpm for 5 min. Thereafter, two immiscible layers were formed, in which the upper layer consisted of mixed hexane with dissolved fatty acid methyl esters (FAME). The upper layer was extracted and dried in an oven at 105 °C to a constant weight. Lastly, 1 mL of the internal standard C17:0 of 0.8 mg/mL in hexane was introduced to the dried FAME and 1 µL of the FAME mixture was then subjected to gas chromatography (Shimadzu GC-2010 plus), with the operating conditions referred to in the literature [3]. Equation (6) was used to calculate the FAME percentages from each attached microalgal cultivation [14].

$$\text{FAME (\%)} = \frac{A_{\text{FAME}}}{A_{\text{ISTD}}} \times \frac{C_{\text{ISTD}} \times V_{\text{ISTD}}}{m} \times 100\% \quad (6)$$

where A_{FAME} is the FAME peak area, A_{ISTD} is the C17:0 internal standard's peak area, C_{ISTD} is the concentration of the C17:0 internal standard (mg/L), V_{ISTD} is the volume of the C17:0 internal standard (L), and m is the mass of the crude biodiesel sample before mixing with the C17:0 internal standard (g)

2.4.4. Analysis of the Net Energy Ratio (NER)

Energy input was an important parameter for assessing the feasibility of the current study for potential use in the industrial sector. The amount of water consumed to produce 1 g of lipid could be formulated using a tailor-made formula, as shown in Equation (7):

$$\text{Water usage per g of lipid produced (g)} = \frac{\text{Amount of water used in cultivation (g)}}{\text{Total lipid content } \left(\frac{\text{g}}{\text{L}}\right)} \times \text{Density of water } \left(\frac{\text{g}}{\text{L}}\right) \quad (7)$$

where the density of water is equivalent to 1 g/mL.

Consequently, the net energy ratio (NER) of the entire process was calculated to be 0.302 by using Equation (8) [15].

$$\text{NER} = \frac{\text{Primary Energy Output}}{\text{Non-renewable Energy Input}} \quad (8)$$

3. Results and Discussion

3.1. Characterization of Palm Decanter Cake

The viability of using palm decanter cake (PDC) as a solid support was closely associated with the elemental content. The PDC was characterized using elemental analysis as being composed of 42.76 ± 2.82% carbon, 6.98 ± 0.67% hydrogen, and 2.78 ± 0.18% nitrogen. The abundance of carbon as a nutrient source proved to be significant for microalgal biomass and lipid production. Generally, the carbon in PDC mainly comprised cellulose, hemicellulose, and lignin [16]. These polysaccharides are external carbon sources to enhance microalgal growth by promoting the uptake of organic materials and microbial photosynthesis. Therefore, microalgal biomass production was strongly escalated [17]. Additionally, proximate analysis was used in this study, which included dry matter and moisture content. The PDC was composed of 98.57 ± 0.22% dry matter and 1.43 ± 0.22% moisture content, vindicating it a ideal for subsequent application as a substrate and carbon source for the production of attached microalgal biomass. In contrast, integrating organic substances with a high moisture contents with the cultivation medium may manifest in bacterial contamination, which would eventually impede the microalgae's growth [12]. The correlation between protein content (14.50 ± 1.80%) and nitrogen content was evaluated by a multiplication factor of 5.3. The C:N ratio in this study was assessed to be 15.38, satisfying the optimum value for outstanding biofilm formation. A C/N ratio of 18 or below is often necessary for appropriate growth and treatment efficiency. On the other hand, values greater than 22 have a negative impact on performance and induce the growth of filamentous organisms [18]. A recent study reported that an optimal protein content of 43% amino-N could be utilized as a building block for microalgal cells to promote proliferation. Overall, it could be claimed that PDC is beneficial as a nutritious carbon source in microalgal cultivation [12].

3.2. Attached Microalgal Growth on Palm Decanter Cake under Various Visible Spectra of Irradiation

The potential of attached microalgal growth on palm decanter cake was studied under various monochromatic and polychromatic visible spectra and continuous irradiation (a photoperiod regime with a light:dark cycle of 24:0 h) (Figure 1). The results could be related to the activity of chlorophyll, as it plays a role in light harvesting during photosynthesis, promoting the growth of microalgal biomass and accumulation of biochemicals [19,20]. In this regard, the maximum microalgal density and microalgal productivity were achieved

under a white light spectrum, namely 1.130 ± 0.06 g/g and 1.213 ± 0.06 g/L per day, respectively. These cultivation parameters were found to outperform other light spectra by at least 5.6%. The white light spectrum resulted in the highest absorption percentage compared with the others, yielding the highest amount of microalgal biomass along with the fastest microalgal growth rate. This implied that the microalgae absorbed most of the photosynthetic active radiation (PAR) from the wavelengths of 400 nm (blue), 500 nm (green), 580 nm (yellow) to 700 nm (red). The amalgamation of the aforementioned colors is responsible for producing the white spectrums, defining the reason why the white spectrum is known as a polychromatic spectrum [21]. However, attached microalgal cultivation under green light produced the least microalgal density (0.623 ± 0.02 g/g) and microalgal productivity (0.655 ± 0.02 g/L per day), due to the fact that *Chlorella vulgaris* is a species of green microalgae in which the pigments or chlorophylls inside the cells are green in color. In this case, the chlorophylls would have reflected most of the green light to make the plant appear green, rather than absorbing it for photosynthesis and cell development. [17]. On the other hand, a previous study proved that the main constituents of *Chlorella vulgaris* are chlorophyll *a* and chlorophyll *b*, of which chlorophyll *a* showed selective absorption at peaks of 440 and 682 nm, while chlorophyll *b* had absorption peaks at 473 and 655 nm [22]. These results indicated that both chlorophylls exhibited blue and red absorption spectrums. Nevertheless, the current research unveiled that the red and blue light spectra contributed to sloughing of the attached microalgal biomasses. The detachment of microalgal biofilms impoverished the attached microalgal density and their ability to thrive on the palm decanter cake's surface. Conversely, the highest biomass production of *Chlorella vulgaris* growing in suspension was measured under red and blue light conditions [9]. These observations could be further rationalized by the use of attached growth versus suspended growth. Accordingly, the longer wavelength of red light was occluded by the presence of the bulk suspended palm decanter cake, preventing it from reaching the microalgal cells due to insufficient photon energy, resulting in stunted growth of the attached microalgae, whereas the blue light with a shorter wavelength had higher energy and could damage the microalgal cells or even lead to cell death.

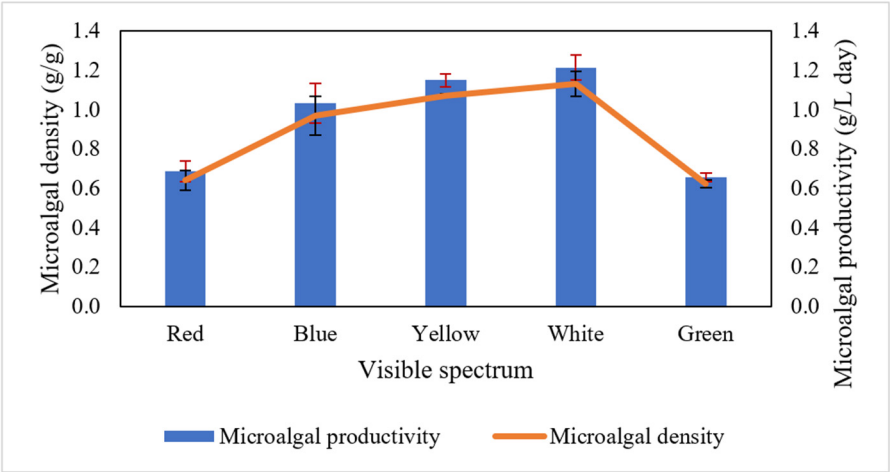

Figure 1. Productivity and density of attached microalgae on palm decanter cake at various visible spectra of irradiation.

According to Table 1, various species of microalgae have tailored optimum spectrums of light colors for cultivation, since each species has its own pigment composition [20]. According to Raqiba and Sibi (2019), red and blue light cultivation could enhance the biomass of *Chlorella vulgaris* compared with other light spectra, which appears to conflict with the findings of this study. However, most of the studies obtained results in line with the current findings, where white light was the best at stimulating the growth of microalgae due to the combined ratio of several light spectra. Although the use of the 24:0 h cycle in

this study may have enabled the attached microalgae to experience a longer light duration in which to perform photosynthesis, the 12:12 h cycle offered by mixotrophic cultivation of attached microalgae permits organic carbon assimilation from the palm decanter cake during the light period, while losing inorganic carbon as carbon dioxide in the absence of light. Consequently, the light would have insufficient time to damage the microalgal cells after penetrating the culture under a 12:12 h cycle.

Table 1. Microalgal growth responses to various visible spectra of irradiation.

Visible Spectrum	Intensity (μmol/m^2 s)	Microalgal Species	Stimulating Effect in Decreasing Order	Reference
White, red, blue, green, yellow	100	*Chlorella vulgaris*	White > yellow > blue > red > green	Current study
White, red, blue, green	60	*Botryococcus braunii*	Red > white > blue > green	[23]
White, red, blue, green, yellow	100	*Chlorella vulgaris* *Diacronema lutheri* *Porphyridium purpureum*	White > red > blue > yellow > green Blue > red > green > yellow > white Green > blue > yellow > red > white	[22]
White, red, blue, green, yellow	- - -	*Chlorella vulgaris* *Tetradesmus obliquus* *Arthospira platensis*	White > red > blue > yellow > green Red > blue > white > yellow > green Red > white > blue > yellow > green	[23]
White, red, blue,	-	*Auxenochlorella pyrenoidosa, Scenedesmus quadricaud, Tetradesmus obliquus*	Blue > red > white	[24]
White, red, yellow	-	*Phaeodactylum tricornutum*	Red > white > yellow	[24]
White, red, blue	52	*Isochrysis galbana*	Blue > red > white	[25]
White, red, blue, green	100	*Chlorella sp., Nannochloris oculata*	Blue > red > white > green	[5]

3.3. Lipid Accumulation from Attached Microalgae Grown on Palm Decanter Cake under Various Visible Spectra of Irradiation

The impact of various visible spectra on the attached microalgal biomass could be further comprehended from the perspective of lipid content and productivity, since lipids are the most important biochemical for the microalgae's downstream processes (Figure 2). Among the five spectra, attached microalgal cultivation under green light had the highest lipid content (14.341 ± 0.14 %) and lipid productivity (93.428 ± 0.18 mg/L per day). In this context, the microalgae favored lipid production and accumulation over growth, as shown by the lowest microalgal density and productivity being found under green light irradiation (Figure 1). This primarily stemmed from the green light being reflected the most, which subjected the microalgal cells to light limitation stress [26]. Accordingly, the microalgae received an inadequate level of photon fluxes for cell growth, thus, inducing the lipid to be stored instead of using them as energy for microalgal cell proliferation. Similar trends were observed for *Chlorella vulgaris* and Ettlia pseudoalveolaris, which both had higher lipid contents when cultivated at intensities of 50 and 150 μE/m^2 per s, respectively, as opposed to a higher light intensity [27]. Moreover, Chen et al. [28] confirmed that Chromochloris zongfingienis under continuous dark cycle cultivation had yielded more lipids than continuous light cycle growth. In fact, any stress that inhibits microalgal growth could convert additional carbon and energy into lipid storage by the cells, predominantly in the form of triacyclglycerols, which are effectively packed in the cells and provide energy for oxidation, thus forming the best reserves for cell reconstruction after the stress condition was removed [28]. On the contrary, red spectrum cultivation accumulated the least lipid productivity due to rapid detachment of the microalgal biomass from the palm decanter cake, which was thus lost to the suspended growth form [29]. However, the lipid content of the attached microalgae under red light cultivation was slightly higher than that of blue, yellow, and white light. The rationale for this was a deficiency in the red light's intensity, as it was unable to penetrate into the culture medium and thus triggered light limitation stress, improving the lipid accumulation of the remaining microalgal cells growing on palm decanter cake.

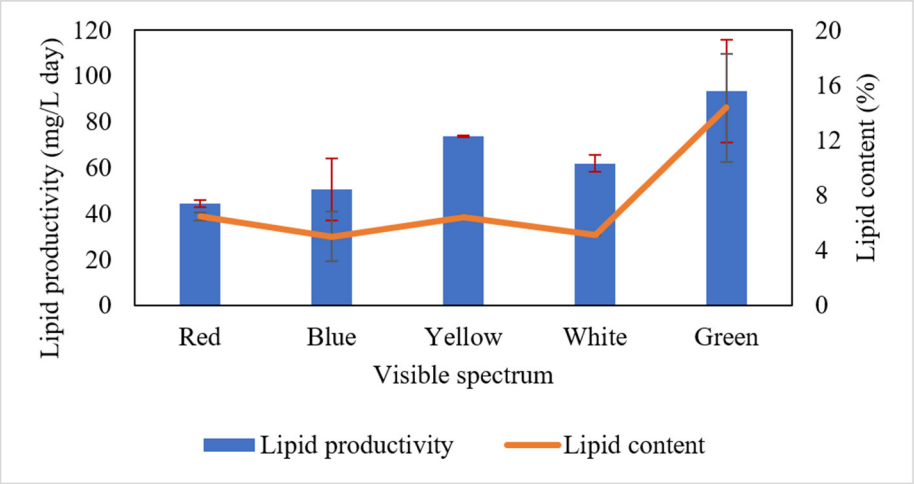

Figure 2. Lipid productivity and contents of attached microalgae growing on palm decanter cake at various visible spectra of irradiation.

Comparing the ability of microalgae to accumulate lipids under various visible spectra of irradiation, Table 2 accentuates that the *Chlorella vulgaris* cultivated under blue light conditions produce the highest amount of lipid [23]. This can be explained by the enzymes within the microalgal cells such as carbonic anhydrase and ribulose biphosphate carboxylase/oxygenase being solely controlled by blue irradiation. Therefore, the presence of blue light enhanced the enzyme activities, triggering triglyceride accumulation in the carbon cycle [30,31]. This earlier finding was contrary to the present study, which showed lower lipid accumulation under blue and white light conditions, considering that the light intensities used here were higher than those in the preceding research. As a result, the photoinhibition process transpired, prompting mutilation of the microalgal cells while degrading the lipid yield.

Table 2. Microalgal lipid accumulation responses to various visible spectra of irradiation.

Visible Spectrum	Intensity (µmol/m^2 s)	Microalgal Species	Stimulating Effect in Decreasing Order	Reference
White, red, blue, green, yellow	100	*Chlorella vulgaris*	Green > red > yellow > white > blue	Current study
White, red, blue, green	60	*Botryococcus braunii*	Red > white > blue > green	[23]
White, red, blue, green, yellow	-	*Chlorella vulgaris*	Blue > red > white > green > yellow	[23]
	-	*Tetradesmus obliquus*	Blue > red > white > green > yellow	
	-	*Arthospira platensis*	Blue > red > white > green > yellow	
White, red, yellow	-	*Phaeodactylum tricornutum*	Red > white	[24]
White, red, blue, green	100	*Chlorella sp.*	Blue > red > white > green	[5]
		Nannochloris oculata	Blue > white > red > green	
White	100	*Nannochloropsis* sp.	Blue > white > blue-green > pink > red	[31]
Red	75			
Blue	15			
Blue-green	20			
Pink	85			
White, red, blue	70	*Streptomyces. bacillaris*	Red > white > blue	[32]
White, red, blue	50	*Chlorella vulgaris*	Blue > white > red	[30]
White, red, blue	104	*Isochrysis galbana*	Blue > white > red	[25]

3.4. Protein and Carbohydrate Contents of Attached Microalgae Grown on Palm Decanter Cake under Various Visible Spectra of Irradiation

Apart from the attached microalgal lipids, other cellular biochemicals, including carbohydrate and protein, were also affected by changes in the visible spectra. Microalgal carbohydrates can serve as a source of bio-alcohols, such as bioethanol and biobutanol [33]. On the other hand, protein from microalgal feedstocks can be extracted for bio-oil, animal feed, medicine, and pigment production [34]. Figure 3 depicts the protein and carbohydrate contents of attached microalgae upon having been exposed to various visible spectra of irradiation. The blue and white light imparted the greatest protein content enhancement (0.341 g/L), and microalgae under white light accumulated the highest carbohydrate content of 0.919 g/L. This trend was inversely proportional to the lipid accumulation (Figure 2), implying that less of the photosynthetic flow of carbon and energy from protein and carbohydrate was converted into the biosynthesis of lipids. Moreover, this could also engender the highest nitrogen uptake from palm decanter cake for conversion into protein, and the highest carbon dioxide assimilation for protein as well as carbohydrate production within microalgal cells. Conversely, green light cultivation accumulated the least protein and carbohydrate content, amounting to only 0.307 and 0.806 g/L, respectively. In this case, the lipids were a better energy reserve than carbohydrate under green light irradiation, i.e., approximately 2.25 times higher in energy value than carbohydrate. Thus, it was shown that green light could trigger the microalgae to build an efficient energy and carbon storage system as opposed to other visible irradiation spectra, offering the greatest sink for available energy accumulation [35].

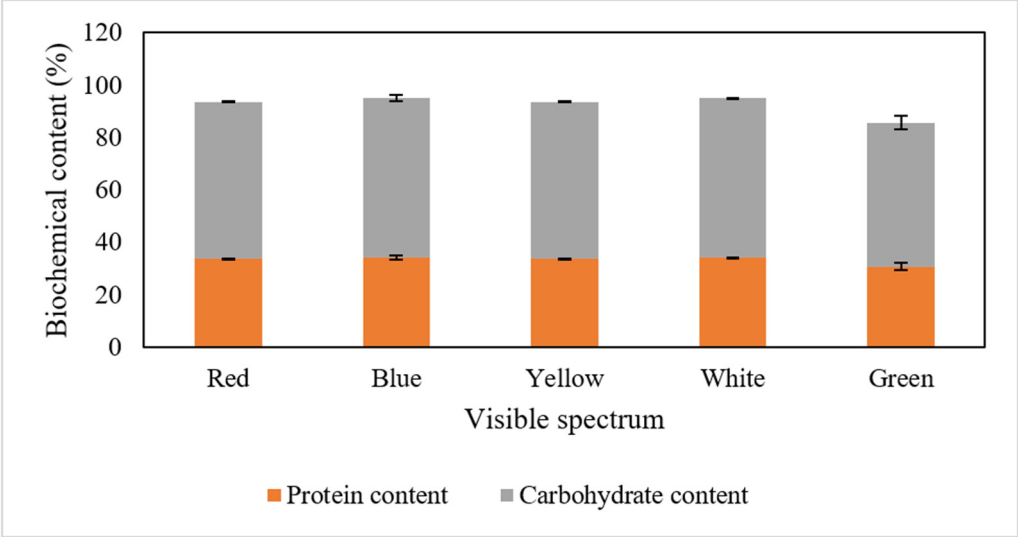

Figure 3. Protein and carbohydrate content of attached microalgae growing on palm decanter cake at various visible spectra of irradiation.

Table 3 shows that the carbohydrate content recorded in the present research was the highest, 0.91 % higher than that found in a study using a glass slide as the surface for attachment. This could be justified by the earlier analysis, which revealed that a bulk material with irregular particle morphologies and tiny particles on the surface of the palm decanter cake was more desirable for microalgae forming an attachment. This was because the rough surface improved the microalgal biomass retention as opposed to the glass slide, which only had a hydrophilic surface [36,37]. As a result, the attached microalgae would thrive by assimilating nutrients derived only from palm decanter cake. Nonetheless, the microalgal attachment on the palm decanter cake experienced protein deficiencies. This was primarily accentuated by the presence of only 2.78% nitrogen content in the palm decanter cake biomass, leading to limited nitrogen uptake. Nevertheless, the fact that the

biochemicals derived from the microalgae populating the palm decanter cake could be improved by optimizing other cultivation variables must not be overlooked.

Table 3. Protein and carbohydrate accumulation derived from attached microalgal biomasses growing on various supporting materials.

Supporting Material	Microalgal Species	Protein Content (%)	Carbohydrate Content (%)	Reference
Palm decanter cake	*Chlorella vulgaris*	34.10 ± 0.65	60.91 ± 1.15	Current study
Spent coffee grounds	*Chlorella pyremoidosa*	20.36 ± 1.13	20.69 ± 0.89	[38]
Cottom, linen, mohair	*Scenedesmus.* sp	50.10 ± 10.1	37.60 ± 10.5	[39]
Walnut shells	*Chlorella vulgaris*	27.70 ± 1.25	34.50 ± 1.31	[40]
	Scenedesmus obliquus	34.80 ± 0.55	32.50 ± 0.59	
Cellulose acetate membrane	*Chlorella vulgaris*	52.84 ± 2.50	37.93 ± 1.15	[41]
Glass slide	Consortia	12.00 ± 1.13	60.00 ± 2.07	[42]
Cellulose acetate–nitrate membrane	*Nannochloris oculata*	19.20 ± 2.59	9.10 ± 0.49	[43]
	Chlorella sp.	19.20 ± 2.57	3.70 ± 0.24	
	Chlorella pyrenoidosa	31.00 ± 2.01	9.00 ± 0.50	

3.5. Biodiesel Profile Derived from Attached Microalgal Grown on Palm Decanter Cake under Various Visible Spectra of Irradiation

Biodiesel, one of the major biofuels produced from microalgal lipid feedstock as a substitute for non-renewable fossil fuel, comprises numerous categories and varieties of fatty acid methyl esters (FAME), which will dictate the quality of the biodiesel. As demonstrated in Figure 4, each visible spectrum for cultivating microalgal biomasses had the highest composition of monounsaturated fatty acids (MUFA), except for white light cultivation, which had the highest content of saturated fatty acids (SFAs) (70.38%). This was possibly a result of the high consumption of MUFAs for microalgal metabolism and growth (Figure 1). MUFAs have a single double bond that is more easily broken down than SFAs, which do not have any double bonds in releasing the energy. On the other hand, PUFAs have been reported to be susceptible to oxidative damage from the presence of reactive oxidative species [9]. The increases in SFAs and PUFAs were proportional to the wavelength of the visible spectrum, implying that the photo-enzymes in the microalgal species were activated by the longer light wavelengths and synthesized more fatty acids, particularly SFAs [30,32]. Less of the excitation energy would be absorbed by the cells to impede photodamage on the acceptors of Photosystem II. Nevertheless, the highest percentage of PUFAs was observed under blue light cultivation as a response to the microalgal enzymes in the cells. It was also discovered that white light produced the lowest biomass (Figure 1) but produced highest SFA and lowest MUFA + PUFA grade biodiesel (Figure 4). This could be because irradiation under white light triggered the stimulation of the photoreceptors, boosting the chlorophyll density and resulting in higher growth. It has also been mentioned in the literature that photochromic stress may sometimes lead to changes in the photosystems, thereby affecting the ultrastructure of the thylakoid organelles in microalgal cells and thus affecting the biodiesel profile [44].

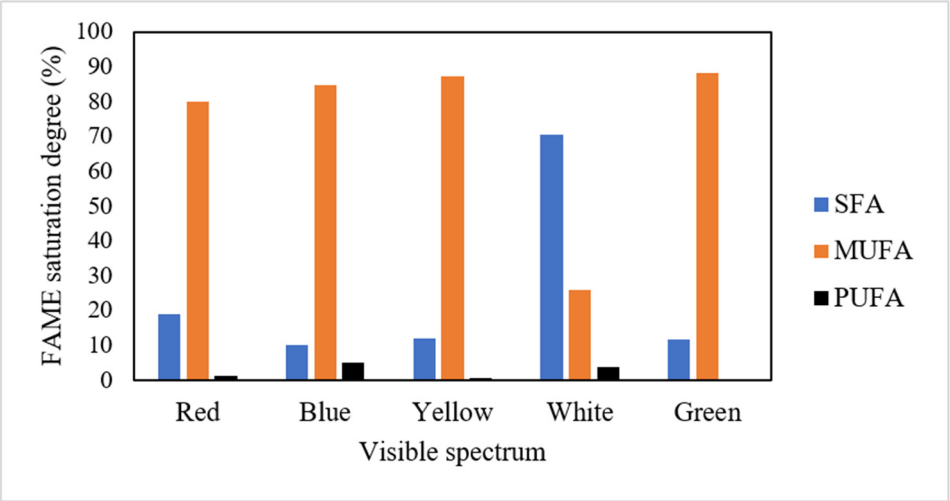

Figure 4. FAME saturation degrees of attached microalgae growing on palm decanter cake at various visible spectra of irradiation.

A study revealed that the general components of biodiesel are C10:0 (capric), C12:0 (lauric), C14:0 (myristic), C16:0 (palmitic), C16:1 (palmitoleic), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), C18:3 (linolenic), C20:0 (arachidic), C20:1 (paulinnic) and C22:1 (erucic) [45]. The FAME species of C16 and C18, namely, palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid are best suited for biodiesel production [2]. Chhandama et al. [1] affirmed that the highest cetane number was discovered in C16:0 and C18:0 fatty acids. This refined ignition quality is needed to ensure cold start performance and reduce the development of white smoke. The longer the carbon chain of the fatty acids and the more saturated the molecules, the higher the cetane number will be [46]. Moreover, biodiesel that was enriched with SFAs would have high oxidative stability against reactive oxidation species, despite having low-temperature properties that could potentially clog the biodiesel fuel filters [2,46]. On that note, the MUFAs such as C16:1, C18:1, C20:1, and C22:1 would produce a superior cold flow. Even though the PUFA-loaded biodiesel exhibited excellent cold flow performance, it was vulnerable to the oxidation process [3]. C18:2 and C18:3 have low melting points which barely form crystals at cold temperatures. This avoids the blockage of fuel filters that is commonly due to the formation of cloudy waxlike structures, and which cause poor engine operation [47]. In the present study, the total SFA content of 70.38%, including C16:0, C18:0 and C24:0, indicates the high cetane number and oxidation stability for preventing the formation of gums, deposits, and sediment (Table 4). The cultivation of attached *Chlorella vulgaris* on palm decanter cake yielded 3.65% total PUFAs, which barely affected the oxidation stability of the biodiesel. Aside from that, this biodiesel also showed moderate cold flow characteristics, as reflected by the low fraction of MUFAs (25.97%) and PUFAs. Since the biodiesel did not contain any C18:2 and C18:3, it was proposed to be blended with other microalgal lipids with a preponderance of PUFAs to enhance the FAME composition and thus better biodiesel quality derived from attached microalgal biomass [48]. A comparative analysis was carried out to evaluate the FAME compositions of various studies that used different microalgal strains cultivated in distinct media, as tabulated in Table 5. It can be noted that the highest degree of SFA was achieved with the FAME profile in the present study, authenticating the exceptional oxidative stability of biodiesel for longer storage.

Table 4. Overall FAME profile derived from attached microalgal biomass grown on palm decanter cake.

FAME Species	FAME Content (%)	Saturation Degree
Methyl palmitate (C 16:0)	51.36	SFA
Methyl stearate (C18:0)	5.36	SFA
Methyl lingnocerate (C24:0)	13.66	SFA
cis-Methyl oleate and trans-Methyl 9-octadecenoate (C18:1)	25.97	MUFA
Methyl cis-11,14,17-eicosatrienoate (C20:3)	1.88	PUFA
Methyl cis-5,8,11,14,17-eicosapentaenoate (C20:5)	1.77	PUFA

Table 5. FAME contents derived from various microalgal strains grown on various supporting materials and growth media.

Microalgal Species	Supporting Material/ Culture Medium	Total C16:0, C18:0, C18:1, C18:2, C18:3 (%)	SFA (%)	MUFA (%)	PUFA (%)	Reference
Chlorella vulgaris	Palm decanter cake	82.69 ± 1.40	70.38 ± 2.30	25.97 ± 1.12	3.65 ± 0.28	Current study
Chlorella vulgaris, *Oscillatoria tenus*, *Scenedesmus obliquus*	Wastewater	78.67	61.57	20.23	11.92	[49]
Nannochloropsis oculata *Thalassiosira weissflogii*	-	44.00 ± 4.30 29.69 ± 3.55	43.03 ± 2.20 26.52 ± 4.65	37.41 ± 3.40 34.25 ± 5.00	15.12 ± 1.30 36.95 ± 4.50	[50]
Chlorella vulgaris	Polyurethane foam	68.70	35.60	43.10	19.70	[3]
Chlorella vulgaris, *Scenedesmus obliquus*	Walnut shells	77.85 ± 5.05 78.67 ± 5.80	32.60 ± 3.96 25.99 ± 2.53	2.85 ± 0.11 15.34 ± 1.60	58.66 ± 5.67 53.66 ± 4.81	[40]
Chlorella vulgaris	Cotton	92.89 ± 0.93	32.37 ± 0.39	28.04 ± 0.40	39.57 ± 0.34	[51]
Dunaliella tertiolecta	-	97.8	32.17	19.45	47.25	[52]
Phaeodactylum tricornutum	-	18.69 ± 3.62	25.77 ± 3.04	31.65 ± 1.94	29.85 ± 5.18	[9]
Ettlia sp.	Porous membrane	90.10	23.60	29.20	43.50	[53]
Dunaliella salina		53.33	34.76	32.18	33.06	[54]
Graesiella emersonni	Vermicompost	82.70 ± 5.49	47.10 ± 2.43	15.30 ± 1.01	37.60 ± 1.66	[55]

3.6. Energy Feasibility of Lipid Production from Attached Microalgal Grown on Palm Decanter Cake under Various Visible Spectra of Irradiation

The evaluation of the net energy by life cycle analysis (LCA) encompassed the microalgal lipid production process, with the proposed flow diagram depicted in Figure 5. The functional unit was assigned as 1 g of lipid extracted from the attached microalgal biomass. Simapro® 8.4.0 was chosen to compute the energy requirements of each process. In microalgal cultivation, water, nutrient, light, and energy inputs were the primary starting concerns for biodiesel production from lab scale to pilot and industrial setups [32]. Figure 6 demonstrates that yellow and white light required the least electricity (0.324 kWh), while green light demanded the smallest amount of water (859.6 g) to produce 1 g of microalgal lipid. This was also supported by the highest lipid content being recorded under green light cultivation (Figure 2). The reduced water usage during cultivation would lead to a lower cost of microalgal dewatering, while minimizing the subsequent wastewater production. Compared with yellow light, which required more water to cultivate the attached microalgae (902.663 g), green light saved an additional 43.064 g of water per 1 g of lipid produced. Since yellow light cultivation led to the highest lipid yield per energy input, the list of energy demands of various processes to yield 1 g of lipid from attached microalgal cultivation is shown in Table 6. In this regard, a net energy input of 115.72 MJ was needed, of which 110.47 MJ (95.46%) stemmed from non-renewable fossil fuels due to the intensive electricity usage. However, the use of palm decanter cake was not incorporated in this assessment, because it was retrieved as a waste by-product to serve a dual role, namely, a

platform and nutritional source for the attached microalgal. Leong et al. [56] claimed that the energy equivalent of 1 g of lipid is 35 MJ.

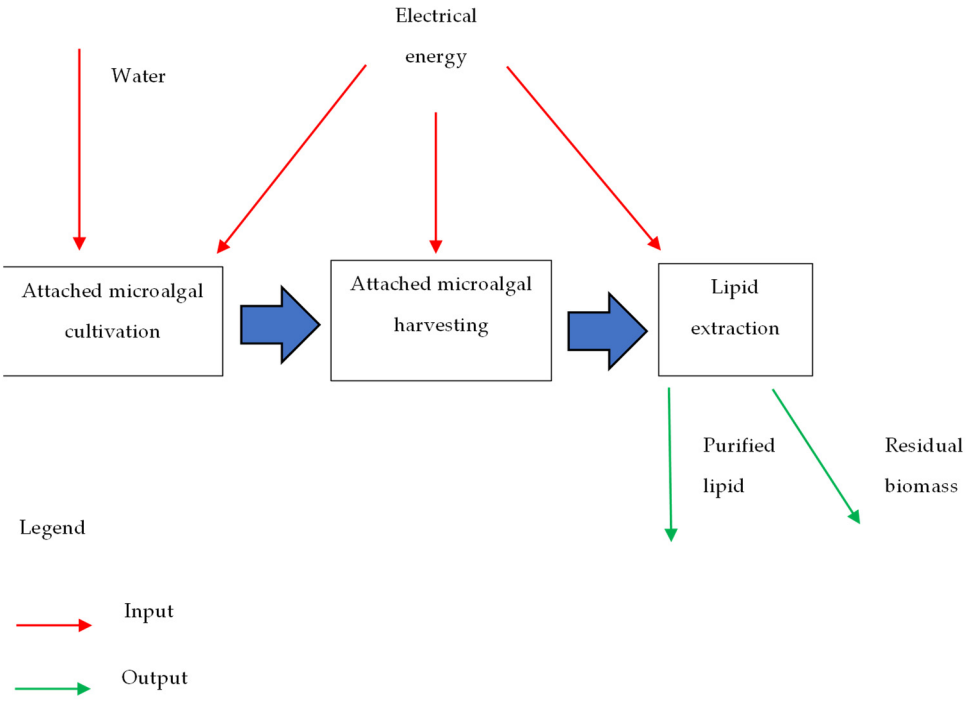

Figure 5. Process flow diagram for microalgal lipid production from attached microalgal growth on a palm decanter cake substrate.

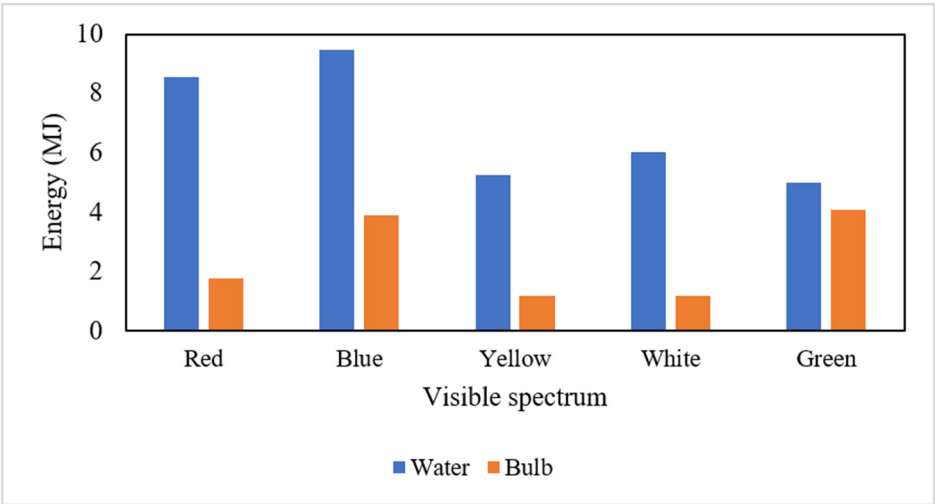

Figure 6. Energy inputs of water and light for growing attached microalgae on palm decanter cake at various visible spectra of irradiation.

Although the system was undesirable because the NER is lower than 1.0, it showed a slight improvement compared with previous research (Table 7) that utilized cool white fluorescent light to irradiate *Chlorella vulgaris* (NER = 0.27) [47]. In this study, light emitting diodes were used, which saved 30% of the power compared with the fluorescent counterpart [57]. Other studies also had NER values lower than 1.0, with the highest being 0.73 for using a mild hydrothermal treatment for lipid extraction that required less electricity (1.78×10^{-4} kWh). This process pumped the microalgal slurry into a reactor at 260 °C and 5 MPa to disintegrate the microalgal cell walls for lipid hydrolysis and solvent-free

extraction in batch reactors for acquiring biocrude for biodiesel production. In a raceway pond, optimistic assumptions were presumed during the cultivation of *Haematococcus pluvialis* and *Nannochloropsis* sp., where CO_2 was supposed to be viable in an external carbon capture system coupled to the biodiesel production process. If the CO_2 from biomass combustion emissions was captured, greenhouse gas emissions would ensue. Even so, this study had the merit of highlighting the generation of microalgal residual biomass enriched with various biochemical compounds such as carbohydrate and protein; hence, this could be further exploited as a feedstock for various industries.

According to the author's knowledge, no comprehensive work has been dedicated to the attachment of microalgae onto solid PDC thus far, other than non-agricultural based inert supports. The utilization of low-cost PDC could concomitantly reduce waste from being generated, with downstream benefits for biodiversity and consumers. The utilization of various visible spectra could overcome the limitation of using natural sunlight to cultivate microalgae by modifying the duration of irradiation contingent on maximum microalgal lipid productivity and lipid yield. In the near future, the photoperiod could be adjusted to compare the microalgal lipid productivity and lipid yield while reducing the net energy ratio. Moreover, the current limitation of this study is caused by the maximal energy utilization of various visible spectra operating 24 h. In real applications, energy consumption can be plausibly reduced by utilizing solar energy during the daytime and visible light during the dark period. This would aid in the operational economic aspects during the scale-up process, materializing the exploitation of microalgal feedstock.

Table 6. Direct processes producing 1 g of lipid from attached microalgal biomass grown on palm decanter cake under irradiation with yellow light.

Microalgae Species	Cultivation System	NER	Reference
Chlorella vulgaris	Attached	0.30	Current study
Chlorella vulgaris	Suspended	0.27	[56]
Consortium	Suspended	0.73	[58]
Haematococcus pluvialis *Nannochloropsis* sp.	Suspended	0.40 0.12	[59]

Table 7. The NER values of different microalgal strains in various cultivation systems.

Process	Amount	Energy Equivalent (MJ)
Cultivation:		
Water	0.9 kg	5.25
Electricity	0.89 kWh	3.20
Harvesting:		
Electricity	26.36 kWh	94.90
Lipid extraction:		
Electricity	3.435 kWh	12.37
Total cumulative energy demand	-	115.72

4. Conclusions

Palm decanter cake (PDC) was found to be a suitable substratum that provided nutrients for microalgal growth and a platform for microalgal attachment. In the assessment of various visible spectrums used to grow attached microalgal biomass, white light was found to be the optimum spectrum for cultivating attached microalgae at a light intensity of 100 $\mu mol/m^2s$ under continuous irradiation conditions. In this context, a microalgal density of 1.13 g/g $\pm$ 0.06 and microalgal productivity of 1.213 $\pm$ 0.06 g/L per day were achieve, along with a high cetane number and oxidation stability in the produced biodiesel. The feasibility of using PDC was further verified by the high protein and carbohydrate contents (34.061 $\pm$ 0.14% and 60.838 $\pm$ 0.15%) derived from mature microalgal biomass regardless of the spectrum. Conversely, the trade-off of utilizing the white spectrum was

the low lipid content and lipid productivity, which could be offset by using green light. Despite that, the improved net energy ratio (0.3) was an indication that non-renewable fossil fuels could be substituted by microalgal fuel to deal with the global warming phenomenon.

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

Abbreviations

Palm decanter cake	PDC
Bold's Basal medium	BBM
Net energy ratio	NER
Light-emitting diode	LED
Photosynthetic active radiation	PAR
Fatty acid methyl ester	FAME
Monounsaturated fatty acid	MUFA
Polyunsaturated fatty acid	PUFA
Saturated fatty acid	SFA
Life cycle analysis	LCA

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