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RESEARCH ARTICLE

Investigations of Light Intensities, Nutrient, and Carbon Sources Towards Microalgae Oil Production *via* Soxhlet Extraction Techniques

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ARTICLE HISTORY

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Abstract: ***Aims*:** This study was carried out to study the optimized condition for microalgae cultivation in terms of light intensity, and nutrient supply. Also, use of a carbon source was studied to optimize the microalgae growth to produce microalgae with a high biomass productivity and a high lipid content.

***Background*:** Algae can be categorized into macroalgae and microalgae. Commonly, microalgae are used to produce biodiesel since microalgae can yield 5000-15000 of oil gallons compared to plant-based biomass as feedstock produced 50-500 oil gallon. Furthermore, microalgae do not face any food crisis and can be cultivated in any wasteland that is not suitable for agriculture throughout the year, compared to crops. Microalgae can also be cultivated in freshwater, saline water and wastewater.

***Methods*:** Microalgae cultivation was carried out with microalgae culture labelled as MX1, MX2, MX3, MX4 and were cultivated under high light intensities, whereas MY1, MY2, MY3, MY4 were cultivated under medium light intensity and MZ1, MZ2, MZ3 MZ4 became control culture that was cultivated under high light intensities and no light condition.

***Results*:** The effect of light intensity, NPK fertilizer, and glucose on microalgae's biomass production will be observed simultaneously. At the end of cultivation, MX2 obtained the highest biomass of 97.186 g. The oil extraction yield is 9.66%. GC-MS analysis showed the presence of UFA and PUFA in the oil.

***Conclusion*:** Thus, future research is needed to improve the technique to increase the microalgae biomass and lipid to become the potential feedstock for the production of biodiesel.

Keywords: Microalgae, light intensity, nutrient, carbon, biofuel, soxhlet extraction techniques.

1. INTRODUCTION

In the last few decades, a trend showed that global transport energy usage increased steadily at a rate of 2% to 2.5%, and the pattern is expected to be continued until 2040 [1]. Hence, the environmental-friendly biofuels are seen as an ideal alternative to replace fossil fuels since the biofuels are produced from renewable sources such as biomass from plant or animal waste.

In the earlier production of biofuel, the feedstock used to produce biodiesel was plant-based oil, which is excellent for the production of biofuels but can lead to a food crisis. Hence, second-generation biofuel exists to combat the issues related to the first-generation biofuel. Nevertheless, the cost of biomass treatment is high and leads to current research, which is the third-generation biofuel derived from algae biomass. Microalgae do not face any food crisis and can be cultivated in any wasteland compared to crops [2].

Commonly, microalgae are used to produce biodiesel since microalgae can yield 5000-15000 oil gallons compared to plant-based biomass. The microalgae need to be cultivated to get a sufficient amount of microalgae biomass, which will undergo lipid extraction to obtain the oil that contained triglycerides. Microalgae can be grown in an open pond system or a closed system with nutrients accessibility, light, pH, and temperature. These factors will determine the amount of biomass produced for lipid extraction [2].

Thus, optimization of microalgae growth is essential to obtain a high yield of oil during the extraction of lipids by adjusting parameters during cultivations to be the replacement of fossil fuels and food-based feedstock for biodiesel. By using renewable energy sources like microalgae, we can curb pollution and fossil foils depletion problems. We can also avoid competition between the biofuel and the food industry.

2. MATERIAL AND METHODS

2.1. Microalgae Identification Contained in the Samples

Two liquid samples were obtained from an aquarium and the pond water. Both locations are located in the Univer-

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siti Malaysia Kelantan area. A light microscope was used to observe the sample. The morphology of the microalgae has been found to identify the species. A previous study was referred that identified the species.

2.2. Microalgae Cultivation in Different Parameters

2.2.1. Effect of Light, Nutrient and Carbon

The microalgae were cultured in containers and filled with 12 L of pond water and 4 L distilled water. Cultures for the extreme light condition were labeled as MX1, MX2, MX3, and MX4 and equipped with eight lamps for every two aquariums. Cultures marked MY1, MY2, MY3, and MY4 were cultivated under moderate light condition and equipped with one lamp for each aquarium. Lux meter was used to measure the light intensity, and average intensity values were being recorded. Aluminum foil was used to fully cover MZ1, MZ2, MZ3, and MZ4. Similar temperature, pH, and nutrients were set for all the cultures, and the air pump was used to provide aeration with a flow rate of 3 L/min. The cultures with the light present were exposed to a continuous light cycle, and observation of the microalgae growth was done continuously for 14 days with three days interval. Container MX1, MY1, and MZ1 were enriched glucose concentration 12 g L^{-1} and 30 g L^{-1} NPK fertilizers as the additional nutrient. MX2, MY2, MZ2 were provided with 30 g L^{-1} NPK fertilizers to measure the effect of NPK fertilizer. MX3, MY3, MZ3 were enriched only with 12 g L^{-1} glucose to measure the impact of glucose on the microalgae culture. MX4, MY4, and MZ4 contained only pond water and distilled water and acted as the control for the nutrient and carbon parameter. The parameter was done simultaneously, and the observation was done for 14 days with three days interval to record the growth of the microalgae.

2.3. Determination of the Number of Cells in the Culture for Cells Growth

Leica camera microscope connected to a computer was used to ease the cell counting process using the hemocytometer. The equation used to count the growth of the average cells was;

$$N = \frac{n}{x} \times 10^4 \quad \textbf{(1)}$$

Where;

N = microalgae density (cells mL-1)

n = average amount of microalgae observed in the square. x= Volume of the counted square

2.4. Microalgae Harvesting

The centrifugal method has been used for microalgae harvesting. The processor used Supra 22K which was equipped with an A250T-6 rotor. Microalgae culture that has the highest biomass was centrifuged for 15 minutes at 5800 rpm. Six bottles with a volume of 250 mL were being weighted using analytical balance before the centrifugation process.

2.5. Cell Disruption Process and Lipid Extraction

After the harvesting process, the microalgae biomass was dried in the oven at 70°C for 24 hours to dry the moisture content of microalgae. Then, the dried biomass was grounded by using pestle and mortar. The microalgae biomass obtained underwent lipid extraction using the Soxhlet extraction method ratio of 1 g of biomass: 3 n-Hexane. The extraction took place for 8 hours at 68°C. Then, the rotary evaporator with a condition of 68°C and 50 rpm was used to separate the solvent phase from the oil for one hour.

2.6. Analysis of Microalgae Oil

2.6.1. GC-MS analysis

Before the analysis, the microalgae oil was prepared to get the purified oil for the analysis. Thus, a Syringe filter with a 0.45µm filter was used to filter the oil. The prepared sample was poured into a 1 mL vial. GC-MS analysis was used to analyze and evaluate the lipid profiles. The microalgae oil analysis was performed using Agilent Tech. G7039A Gas Chromatography-Mass Spectrometry machine. The column used in the study was a fused–silica capillary column (30 mm x 0.25 µm, i.d., thickness 0.10 µm). The column flow rate was 1.0 mL/min. The initial temperature was set at 50 °C and was held for 5 minutes. The heating rate was 15 °C before it reached the final heat, which is 300 °C, and the hold time was 15 minutes.

2.7. Statistically Analysis

Two-way ANOVA and student's t-test was carried out using to test the significant difference between the parameter using IBM SPSS Statistic 25. Statistical analysis using two-way ANOVA was done to study the interaction between light condition and nutrients present in the culture. The student's paired t-test was done between light condition and no light condition.

2.8. Oil Extraction

The biomass recovered during harvesting was 97.186 g for the first batch of MX2. The second batch cultivation with a similar condition as MX2 obtained 97.851 g in the cultivation. Fig. (**6**) shows the microalgae biomass that has been harvested. The highest lipid extraction yield produced by microalgae was 9.66%.

2.9. Wet Paste Microalgae VS Microalgae Dry Powder

97.186 g of the microalgae biomass, which is in a wet paste form, is being extracted and produced in a greenish color in the solvent. Then, after evaporation,10 mL of clear liquid was obtained, which shared characteristics like water, as illustrated in Fig. **7**, and no oil was achieved because a moisture content in the biomass was extracted along with other components.

The microalgae powder was extracted using Soxhlet extraction and produced a yellowish-greenish solution compared to microalgae in the wet paste form. Fig. **7** shows the result of evaporation. The oil extraction yield is 9.66%.

2.10. GC-MS Analysis

Based on the result obtained, Eicosadienoic acid, methyl ester (C20:2n-6c,9c), and Octadecenoic acid (C18:1) were the fatty acids found in the microalgae oil. As for the result of this study, the microalgae oil showed a frequent area of C18:1, which was 36.45% and had 83.92% of the score, indicating the potential of microalgae oil to become feedstock for the production of biodiesel. In this study, 3.04% is the (C20:2n-6c,9c) which is categorized as polyunsaturated fatty acid (PUFA).

3. RESULTS

3.1. Microalgae Identification

Figure **1** and **2** show the difference in the type of microalgae contained in both samples, observed under a light microscope. In 0.05 ml of the sample, the result showed that pond water has more microalgae species than aquarium water, as shown in Fig. **3**. The species in the pond water were recognized as *Scenedasmus sp.* (S1), *Monoraphidium sp.* (S2), and *Chlorella sp.* (S3). The aquarium water only contained *Scenedasmus sp.* (S1) [3].

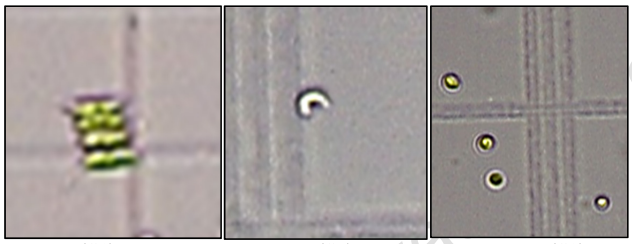

Fig. (1). Species found in both sample, *Scenedasmus sp.* (S1), *Monoraphidium sp.*(S2) and *Chlorella sp.* (S3). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

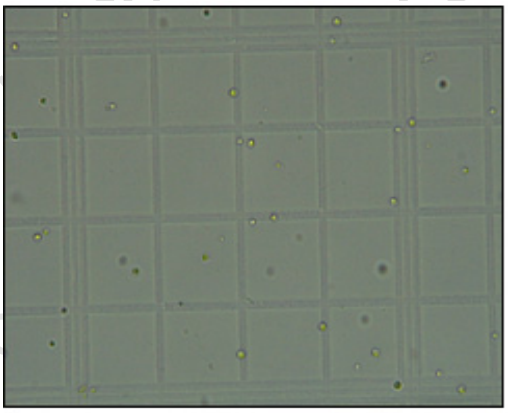

Fig (2). Microscopic picture of MX2 at day 12. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.2. Growth Study of Microalgae in Different Parameters

3.2.1. Effect of Light

Fig. **4(a)** shows the growth curve of MX1, MY1, and MZ1, and the highest growth curve at the early stage belongs to MX1 that has been exposed to 5603.33 lux light, *i.e.* the highest light intensity in the experiment. MX1 also does not show any lag phase compared to MZ1 culture. However, MX1 growth started to drop drastically on day six and continued to decline until day 14, probably because of the over-exposure of light and the photoinhibition of MY1 that was exposed to 336.76 lux. It shows that the culture growth undergoes an early exponential stage compared to MZ1. However, the increase in MY1 is still lower than MX1 since the amount of light provided was different. The new growth of culture starts after the death phase on day 12 because of little competition of nutrients and light sources. Nevertheless, both MX1 and MY1 undergo the early death phase compared to MZ1, which is related to the presence of nutrient and carbon sources. The results also show that the photoinhibition occurred in both cultures. As for MZ1, the growth fully utilizes the nutritional sources that are present in the culture since it does not have light sources, but the increase is slower than MX1 and MY1.

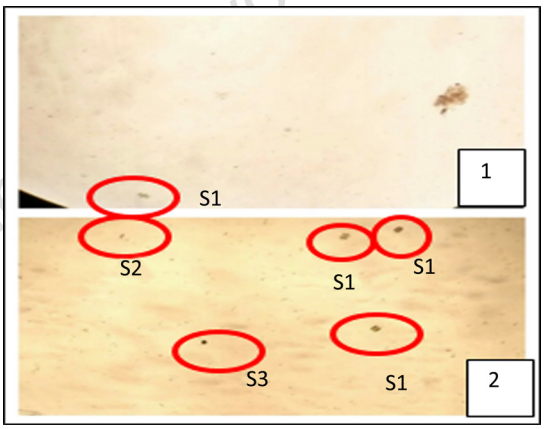

Fig. (3). Microalgae species and amount of biomass contained in both samples. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Fig. **4**(b) also indicates that MX2 has the highest cell growth since it received the highest light intensities. The graph shows that microalgae that have been receiving extreme light with the aid of nutrients can produce high biomass. Even at the death phase, the amount of biomass contained in the culture is still the highest compared to MY2 and MZ2. The culture also does not encounter the early death phase, like MX1. The death phase-only occurred at the end of the cultivation, which indicated that the photoinhibition does not happen during the cultivation phase and only happened at the end of cultivation. MY2 has lower light intensity compared to MX2. Thus, the culture shows that the microalgae biomass decreases drastically after day three and

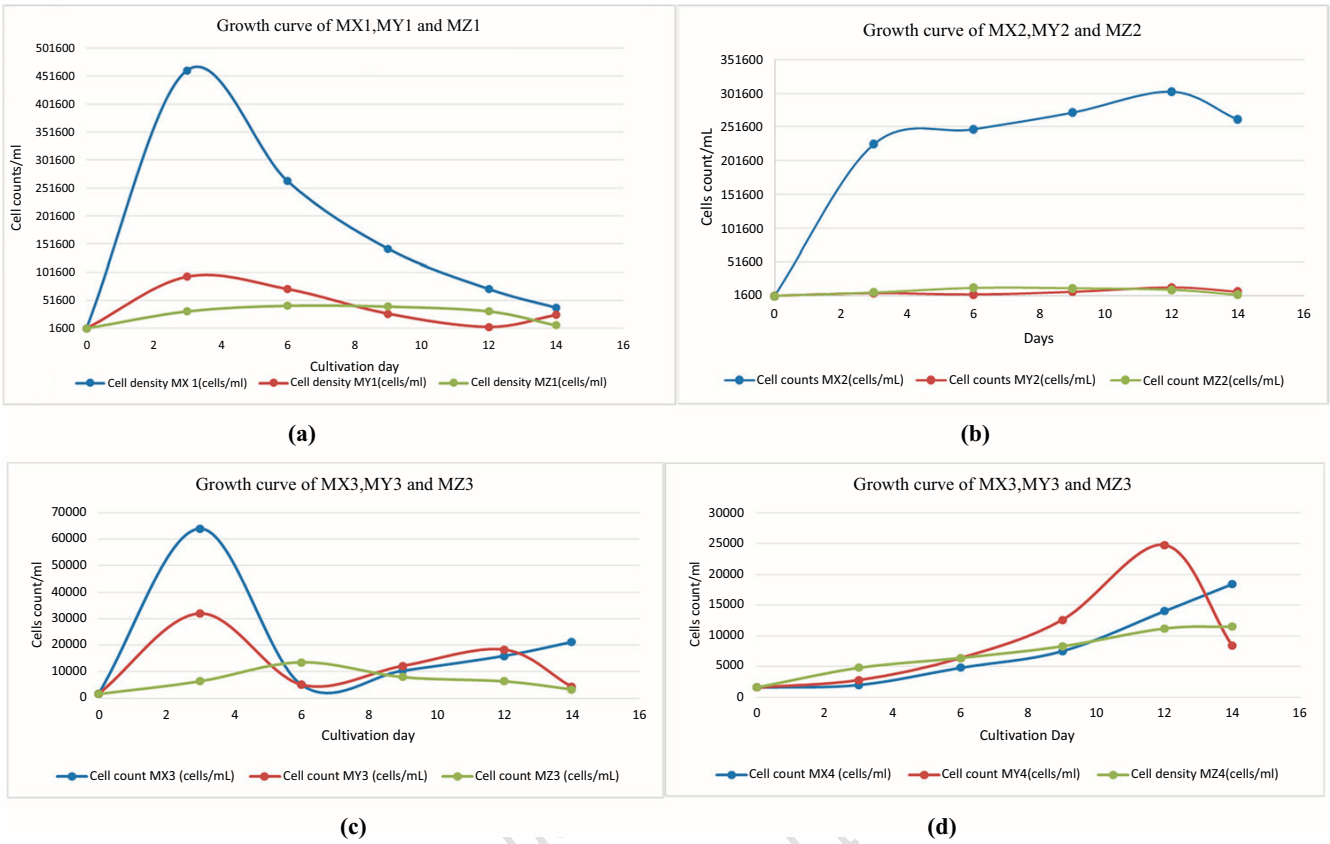

Fig. (4). The growth curve of cultures **(a)** MX1, MY1 and MZ1, **(b)** MX2, MY2, and MZ2, **(c)** MX3, MY3, and MZ3 and **(d)** MX4, MY4, and MZ4. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

encounters the early death phase on day six. Despite the early death phase, the culture does not show any lag phase and stationary phase.

Nonetheless, after day 10, the cells started the new cycle and increased gradually until day 12. The cells' growth began to decline at day 12 as the nutrient might be depleting. Similar to MZ1, the growth of MZ2 wasgradually increased, but it was the slowest compared to MX2 and MX2. MZ2 culture used heterotrophic mode, which utilizes the NPK fertilizers for microalgae productivity.

Fig. **4(c)** illustrate the growth curve of cultures with the presence of glucose in three different light intensities, which are labeled as MX3, MY3 and MZ3. Similar to other cultures, the highest growth curve belonged to MX3 since it was cultivated under extreme light compared to the other two. Nevertheless, on day 6, the culture already encountered the death phase, probably due to excessive sources that the microalgae obtained in terms of light and carbon. But, the new cycle started quickly since it was still exposed to extreme light conditions until the end of cultivation. MY3 has a similar growth pattern to MX3 except for day 14, in which the curve indicates the death phase of the cultures due biomass covered the top of the cultures. The increase of biomass on day six was because of low competition for the sources among cells. For MZ3 culture, the growth curve still has a noticeable improvement since it is supplied with glucose. Hence, the microalgae use glucose in the metabolism pathway to do cell proliferation to increase the number of biomass.

Fig. **4(d)** shows the cell count with glucose sources for MX4, MY4, and MZ4. The cultures were not enriched with any additional nutrient sources and relied solely on the light. MX4 shows slow growth and encounter lag phase, despite cultivated under extreme light intensities since it was not supplied with external sources compared to cultures with the same light condition. Nonetheless, culture has a longer growth curve compared to MX1, MX2, and MX3. MY4 also showed a slower growth compared to MX4, but the cultures underwent a late exponential phase, which was on day 12. However, the death phase immediately happened probably because of high competition for nutrients since the microalgae culture obtained the highest biomass on day 12. MZ4 has the fastest microalgae growth compared to MX4 and MY4 by utilizing the atmospheric CO_2 to grow.

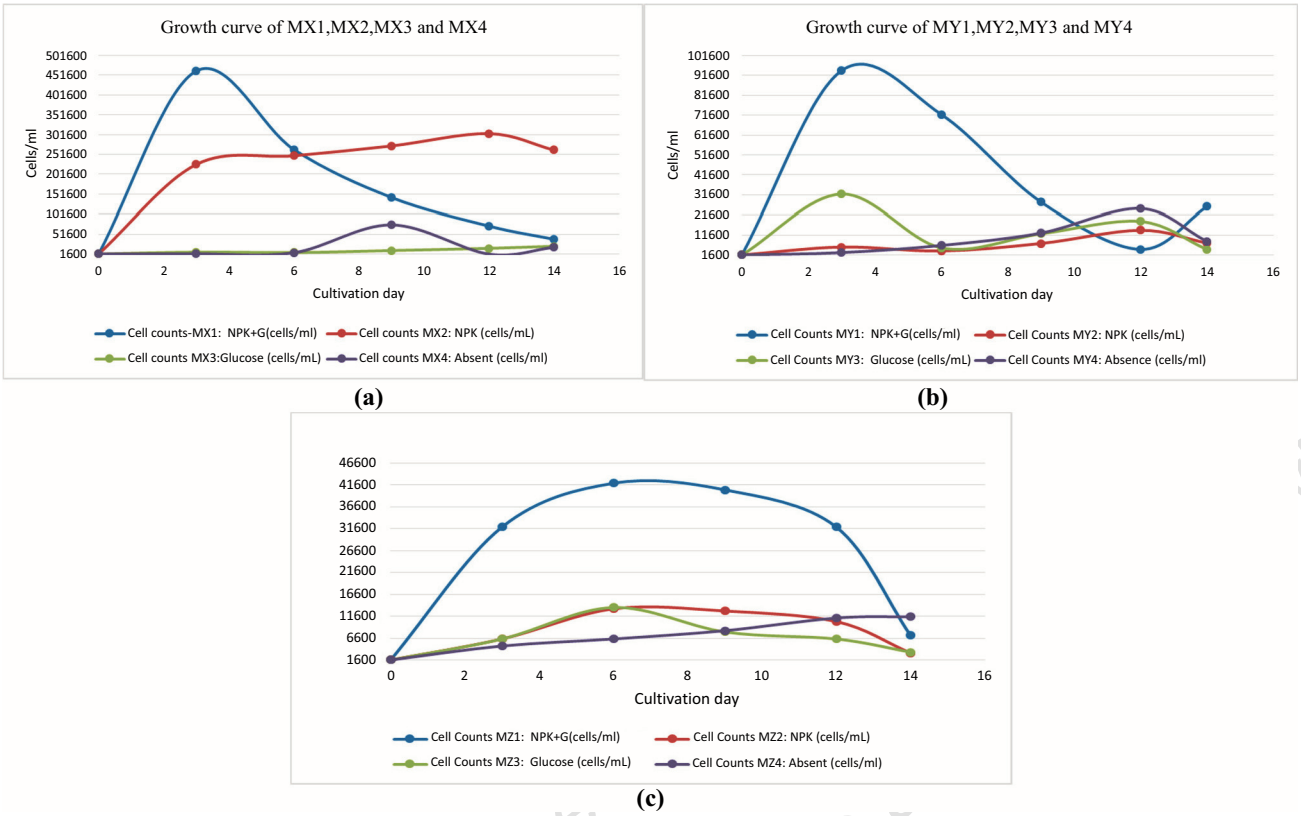

Fig. (5). The growth curve of cultures **(a)** MX1, MX2, MX3 AND MX4, **(b)** MY1, MY2, MY3 and MY4 and **(c)** MZ1, MZ2, MZ3 AND MZ4.

3.2.2. Effect of Nutrient and Carbon Sources

Fig. **5(a)** shows the difference in the growth curve between MX1, MX2, MX3, and MX4. MX1 and MX2 have a higher growth compared to MX3 and MX 4 at day 0 to day 3. The maximum biomass density can be seen on day 3 by MX1 since it is supplied with the presence of NPK fertilizers and glucose with the aid of extreme light conditions compared to other cultures. However, since the culture has been put under high light intensities, the photoinhibition was likely to happen, and the abundance amount of additional nutrient sources also inhibited the cells from growing, resulting in the growth declin on day 14. From Fig. **5(a)**, MX2 shows that the growth increases steadily and reached maximum growth at day 12 with 304000 cells/ml. The drastic growth in the first three days of cultivation was because of NPK fertilizers, and it contributed to rapid cell growth and frequent cell division, and the growth continued until the day 12. After day 12, the microalgae started to undergo the death phase, which cells are likely to die, which we assume the concentration of NPK fertilizer is decreasing at the end of the cultivation [4]. MX3 culture contained only glucose in which the growth curve showed a slow growth from day zero to day six, and the growth encountered the late exponential phase after day six. Yet, the culture still indicated a positive growth, which could be concluded saying that the microalgae use the mixotrophic mode in the cultivation. Thus, the combination of light and glucose sources can stimulate the growth of microalgae [5].

Fig. **5(b)** shows the growth curve for MY1, MY2, MY3, and MY4, which have lower light intensities than MXs culture. However, the condition for each culture in terms of NPK fertilizer was similar to MXs culture. MY1 has the highest biomass productivity at the early stage of the cultivation before it started to decrease gradually, indicating the death phase in the culture. The new cycle started because of a low number of cells and little competition for nutrients. MY2 cultures also have noticeable growth at the early stage of cultivation.

Similar to MY1, the death phase also started earlier, which was on day 6. In the cultures, the presence of NPK fertilizers does not have a significant impact on the amount of biomass produced by microalgae compared to MY1 and MY3. However, after day 6, the cultures started to growwhich was assumed due to the presence of light in the cultures. Positive growth has also been shown by MY3 cultures, glucose-enriched culture with the aid of low light. The death phase also happened earlier in this culture due to the depletion of glucose. Similar to MY2, cell proliferation start-

ed after the death phase since the cultures still have light sources as the energy supply for growing. Compared to other cultures with the same light condition, MY4 was entirely dependent on a light source that has been supplied since it does not have any additional nutrient sources, which explained the slow growth of the cultures. Nonetheless, the growing phase was longer than other cultures treated under the same light condition.

Fig. **5(c)** shows the growth curve for MZ1, MZ2, MZ3, and MZ4. Aside from not supplying with light sources, each culture received the NPK fertilizers similar to MXs culture and MYs culture. The highest cell density was produced by MZ1, which has NPK fertilizers and glucose compared to the lowest growth curve, which is MZ4 since it does not enrich with additional nutrient sources. Compared with MX1 and MY1, the production of microalgae in MZ1 is lower since the light intensity also needs to be considered in microalgae cultivation. However, compared with the cultures under the same light condition, the culture has the highest growth at the initial cultivation stage.

Nonetheless, at the end of the cultivation, the culture growth also dropped drastically, similar to MX1. MZ2 cultures undergo an early exponential phase since it does have NPK fertilizers to boost the microalgae growth, which has the same growth pattern as MZ1. For MZ3, the growth was steadily increasing, which raised at the earlier stage (exponential phase) but have a constant decrease until day 14 (death phase). The continuous growth of MZ3 culture must be contributed by the glucose as the carbon source to enable the microalgae to do the heterotrophic mode since no light was provided to the culture. At the end of the cultivation, the culture that obtained the highest biomass, *i.e.* MX2 culture which contained NPK fertilizers and has been cultivated under the extreme light intensity, was chosen to be harvested.

3.2.3. Statistically Analysis

Based on a two-way ANOVA result, there was a statistically significant interaction between light intensity and presence of nutrient sources with the cell count of the microalgae biomass, which at p=0.002, and the p-value is 0.002. There are also significant differences between the condition of light, which was $p<0.0005$, and the result was also applied to the presence of nutrient sources since it shows significant differences between the presence of the nutrient sources at $p = 0.002$.

The student's t-test for light intensity also has been done between extreme light conditions and even the no-light condition. Since the Sig. (2-tailed) value is 0.005, which is $\leq$ 0.005, we can conclude that there was a significant difference between the mean number of cells count between these two conditions. Hence, there was a significant difference in the amount of microalgae cells count for extreme condition light (91966.67 ± 132965.715) and the control condition, which is no light condition (11741.67 ± 12219.475) conditions; t:46=2.943,p=0.005.

4. DISCUSSION

Based on results, it shows that pond water contained more microalgae biomass compared to the aquarium sample, due to the locations of the pond that is more suitable for microalgae cultivation since it is exposed to sunlight and atmospheric carbon dioxide. The ponds also received nutrients from excessive fertilizers from the nursery since it flowed into the pond water. In contrast, the aquarium location has a limited light source, and it was not enriched with any additional nutrients [6].

Generally, low light intensities cannot provide enough amount of light for the microalgae cells for carrying out the photosynthesis and the cell proliferation since the light cannot penetrate the dark areas that have been covered by the microalgae cells. Thus, cells in low light intensities have lower cell counts than in extreme light. As for the control culture, which is the dark condition, all the cultures show the slowest growth compared to the other two conditions. The cultures in no light condition use a heterotrophic mode in which the microalgae will utilize the nutrient present in the culture and the carbon sources. Thus, the growth was much slower than the cultures that have been placed under fluorescent lamps. A previous study stated that lower light intensities and temperatures would slow down the growth of microalgae [7].

In the study of nutrients and the carbon present, from all the result shown, MX1. MX2, MX3, MY1, MY2, and MY3 proved that additional nutrient and carbon sources, as well as cultivation in the presence of light sources, can increase the number of biomass in a shorter time. All the cultures will show a drastic increase in the biomass at the early stage of the cultivation compared to cultures that do not have additional nutrient sources. The presence of additional nutrients boosts the growth of microalgae. Carbon sources can also influence the growth of microalgae, especially during the heterotrophic mode, which only utilizes organic compounds without light sources. Thus, in this experiment, glucose is used to provide carbon sources to the microalgae. Glucose mainly enhanced *Chlorella kessleri sp.* under mixotrophic and heterotrophic conditions. Although glucose increases the microalgae productivity, it also triggers the growth of filamentous fungi. However, the growth of filamentous fungi and microalgae can increase the production of lipid as filamentous fungi can synthesize up to 46.6 of lipid [8, 9].

Literature data show that a pH value of 8.5 improves the microalgae growth in the presence of bicarbonate as the carbon sources. The result shows the *Synechoccous* growth improved when the pH reached 8.5. However, the production of biomass decrease. Thus, the best condition for microalgae cultivation is at pH 7.7 which obtained 6 g L^{-1} of dried biomass for 250 mL culture [10].

Based on the two-way ANOVA, it can be concluded that there was a statistically significant interaction between light intensity and presence of nutrient sources with the cell count of the microalgae biomass, which is F(6,60)=3.975,p=0.002. Thus, the cultures which have both light source and addition-

al nutrient sources can contribute to high microalgae biomass production since both light and nutrient can aid the microalgae growth compared to cultures that only depend on atmospheric condition. As for t-test between no light condition and the presence of the light, we can conclude that light intensities play a significant role in microalgae growth.

During the extraction process of the wet paste, due to the limitation of feedstock, low oil was extracted. Based on previous studies by Sharmin *et al*. (2016) showed that the oil recovery for n-hexane by wet biomass is 2.49%, which differs from this experiment since the extraction produced water--like liquid as shown in Fig. (**6**). This probably is due to the number of lipids contained in the microalgae or depends on species that are used in the extraction process [11]. As for extraction using microalgae powder, it produced a yellowish-greenish solution compared to microalgae in a wet paste form, as seen in Fig. (**7**). The oil extraction yield was more significant than the one by Tantichantakarun *et al*. (2019) who obtained 5.52% [12]. However, the oil yield was low for biodiesel production probably because Soxhlet extraction is not suitable to extract some species contained in the culture since the cell walls of the microalgae species are thick and hard to disrupt, such as *Chlorella sp.*

Fig. (6). Microalgae biomass after the centrifugation process. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

For microalgae analysis, the previous study found that it is common to find saturated fatty acid in microalgae as shown in Fig. (**8**). The result of this study is consistent with the statement made in the last study since it consists of C20:2n-6c,9c, and C18:1. Besides, the composition consisted of C16-C20 which is suitable for the production of biodiesel. Moreover, a saturated fatty acid commonly gives a better impact on biodiesel and also crucial in biodiesel production. The PUFA found in the oil can be used in other areas such as cosmetics fields since PUFA is commonly known to have a higher oxidation value compared to a monosaturated and saturated fatty acid [13, 14].

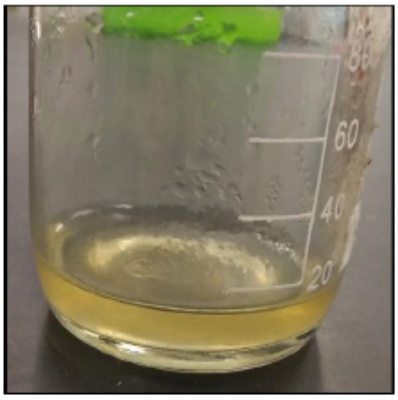

Fig. (7). Moisture extracted from the wet paste biomass. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

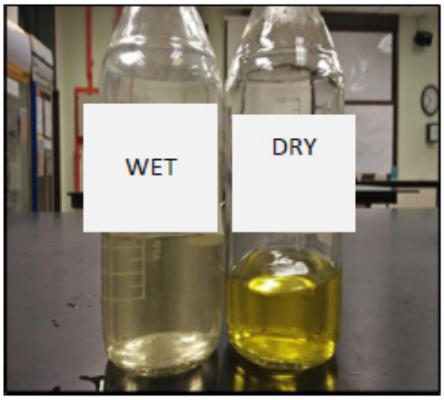

Fig. (8). Comparison between wet route and dry route extractions. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

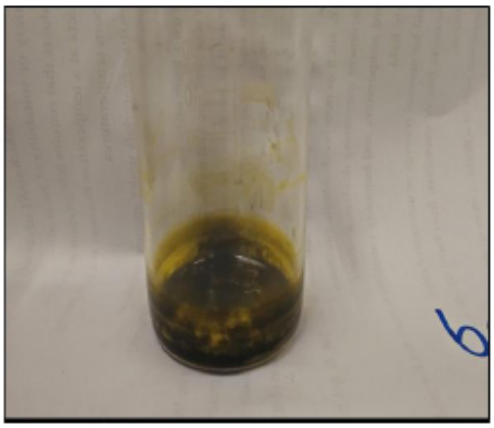

Fig. (9). Extracted oil obtained from microalgae biomass in the powder form. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

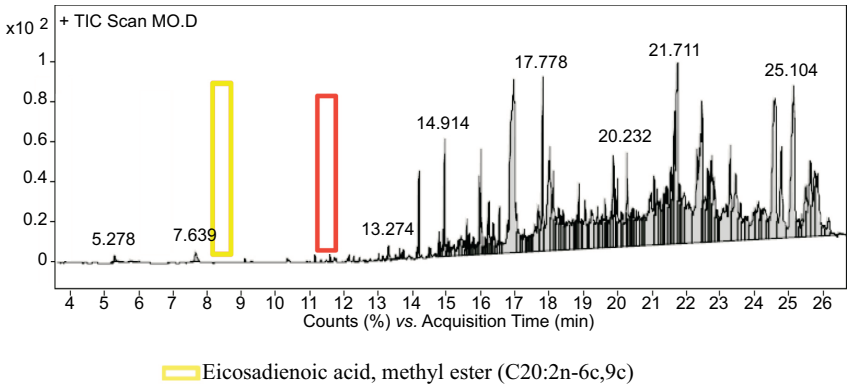

Fig. (10). GC-MS extractable profiles of microalgae oil extracted using Soxhlet extraction. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Stearic (-octadecanoic-C18:0) is one of the fatty acids that is based on the microalgae oil and found at the accumulation of lipid in lag or stationary phase. However, both saturated (SFA) and unsaturated (UFA) microalgae oils should be a balance in the composition to produce biodiesel that has a high quality. However, the studied microalgae oil does not show any unsaturated oil in the composition because of the growth phase during cultivation or the nutrient provided during cultivation that affects the content of lipid in microalgae (Fig. **9**) [15, 16].

During the cultivation process, the microalgae cultures faced the death phase or crash culture, which affected the amount of microalgae production. The culture crashes can be caused by a variety of factors such as the depletion of nutrients, pH disturbance, or contamination. Microalgae crashes can be controlled by applying a small dose of pesticides and antibiotics to control contamination. Suitable concentrations of nutrients are needed to increase the microalgae.

Humphrey *et al*., 2019 stated that the microalgae cultivation process could also be improved by developing a better empirical model that gives an accurate description of cultivation compared to the Logistic and Manod models for growth (Fig. **10**). It was also found out that magenta light is better than other light sources such as sunlight and white light at high intensities [15, 17, 18].

CONCLUSION

For prospects, it is necessary to study the best species of microalgae that have the highest cell productivity to get a higher biomass in a shorter time. It is also essential to know which day is suitable for harvesting to get increased extracted oil production since the stationary phase produces the highest oil yield during extraction. Direct extraction from wet microalgae biomass should be investigated as it can be both cost- and time-effective.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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