PRELIMINARY EXTRACTION OF CHLOROPHYL A AND B FROM MICROALGAE ISOLATED FROM SELANGOR SEA WATER

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Abstract

Microalgae are organisms found in marine and freshwater environments. This organism is a natural source for a variable valuable compounds such as carbohydrates, proteins, lipids as well as pigments. Method for extraction of pigment from microalgae will be varied depending on the type of microalgae. The objective of this study was to obtain the chlorophyl *a* and *b* pigment from the two microalgae isolated from marine sample in Selangor. Several factors were tested to maximize the yield of pigments extraction, such as the solvent to be used, the cell wall disruption technique, the extraction time and the use of different empirical correlations

This study proposed to extract Chlorophyll *a* and *b* pigment from the sample by using methanol (99.5%), acetone (99.5%), and ethanol (95%) as the extraction solvents beside physical disruption using glass bead. Spectrophotometric analysis has been used to analyze and quantify the pigments. Chlorophyll *a* and chlorophyll *b* content of acetone extracted samples were higher than compared to ethanol and methanol. For sample 1, the results were given as 3.39 µg/mL and 2.80 µg/mL for chlorophyll a and chlorophyll b, while for sample 2, the results were given as 3.30 µg/mL and 2.95 µg/mL respectively.

Keywords: Microalgae; pigment; chlorophyll a; chlorophyll b; extraction

Introduction

Microalgae, are unicellular which consists of both prokaryotic and eukaryotic. This aquatic algae will be found in freshwater and marine systems which is 1-50 micrometer in diameter without any roots or leaves (Singh, Liu, & Sharma, 2013). According to an estimation about 200,000 to 800,000 species in many different genera exist of which about 50,000 species are described (Marilum, 2019). Over 15,000 novel compounds originating from algal biomass have been chemically determined. Microalgae contain proteins, sterols, vitamins, pigments, carotenoids, and polysaccharides that can be extracted for further applications such as biofuel production, pharmaceutical, food and cosmetic industry. Microalgae has various types such as blue green algae, green algae and red algae. They are both harmful and useful. Algae can tolerate and grow in different environment, temperature, with or without light, in fresh or salt water.

Currently researches on microalgae biotechnology are still in progress in Malaysia. Since we are having rich microalgae resources, researches are tend to find ways to produce high-value products. The climate in Malaysia is more favourable to grow microalga as the sun shines all year round. Producing natural pigments, environmental friendly and biodegradable pigments can replaces the toxic and hazardous pigment to human health. Algae consists of three major classes of photosynthetic pigments which are chlorophylls, carotenoid (carotenes and xanthophyll) and phycobilins. A primary pigment in microalgae which absorb blue and red wavelength and then transmits green light is chlorophyll. Environmental factors such as light intensity, temperature, pH and nutrient presence in culture medium affect the pigment production in microalgae (Begum *et al*., 2016). Suitable medium is needed to culture the microalgae. For the light intensity, sunlight or artificial light source can be used in culturing microalgae. Chlorophyll is one of the valuable bioactive compounds that can be extracted from micro algal biomass. Chlorophyll is an essential compound in many everyday products. It is used not only as an additive in pharmaceutical and cosmetic products but also as a natural food colouring agent. Chlorophylls and its derivatives are essential as pharmaceutical and also is being used in

cosmetics. They have some wound healing and anti-inflammatory properties so that they are being used widely in medicines (Ghosh *et al*., 2018). Chlorophylls have a wide range of applications due to its coloring effect, tissue growth stimulating effect, antioxidant and ant mutagenic properties (Halim *et al.*, 2010).

Extraction solvents such as methanol (99.5%), ethanol (95%) and acetone (99.5%) were widely used in extraction of pigment from microalgae. As cell disruption will likely play an essential role in the successful extraction, including using glass beads while vortexing the sample. The degree of affinity to the chemical composition of the pigments is depends in choosing the effective solvent. The objective of this research is to study the best method to extract chlorophyll a and chlorophyll b pigments from microalgae isolated from Selangor. In addition, spectrophotometric method is the most useful tool to do photosynthetic pigments analysis. With specific wavelength the optical density is identified to evaluate the pigments.

Materials and Methods

Strain and culture conditions

Two different microalagae, microalgae 1 and microalgae 2, were obtained from Unisel Culture Collection. Stock cultures were kept in falcon tubes under 1000 lux of light intensity and always maintained with temperature of 16°C to 19°C.

Cultivation of microalgae

The stock cultures were used in cultivating process in 250 mL of Erlenmeyer flask containing 90 mL BG-II medium added with 10 mL of inoculum. The stock culture were handled in sterilized condition to avoid any contamination. After inoculation, the algae cultures were placed under artificial light intensity with 1000 lux with connected air pump and sterilized air tube. The air pump created aeration and the sedimentation of the algae was prevented by mixing (Maurya *et al*., 2014). The temperature was maintained to 37 °C.

Extraction of pigment

The growth of microalgae was monitored regularly so that the microalgae were harvested when the pigment content was the highest at the end of stationary phase (Ilavarasi *et al*., 2011). 5 mL sample culture was withdrawn into 50 mL falcon tube. The cells were centrifuged at 7000 rpm for 5 minutes. The upper part which is supernatant was removed. Before the centrifugation, the empty 50 mL of falcon tube was weighed. The pelleted cells contained falcon tube was weighed after the centrifugation. Optimization of extraction process was carried out using different solvents which were methanol (99.5%), acetone (99.5%) and ethanol (95%). The pellet was suspended in calculated volume of solvent by strong vortex mixing for 20 minutes. After the strong vortex mixing at high speed, the mixture of cell and solvent was centrifuged at 3000 rpm for 1 minute. The supernatant was collected for further spectrophotometric analysis.

Spectrophotometric analysis

In spectrophotometric analysis different pigments from microalgae absorb different wavelengths of light between range of 400 nm and 700 nm. As chlorophyll *a* and *b* are the main pigments in microalgae the chlorophyll absorbs blue and red wavelength and transmits green light. Quantification of the pigment extracted was carried out by using composed equations (Henriques *et al*., 2007). Finally, statistical analysis was analysed by one-way ANOVA (analysis of variance). The means comparisons significance was tested at $P<0.05$.

Results and Discussion

Regular observation was carried out frequently to ensure the growth of microalgae. After 5 days of the inoculation, the microalgae culture was turned to be green in color. Based on the optical density measurement, growth rate of the both microalgae gradually increased

from 5th day to 25th day. Equations were proposed to determine the concentration of the pigments by using specific absorption coefficient (Lichtenthaler, 1987).

Organic solvents were used in extraction of pigments from microalgae sample. Table 1 show the concentration of the pigments extracted by three different solvents which determined by using the composed equation for chlorophyll a, chlorophyll b and total chlorophylls.

Table 1: The concentration of chlorophyll *a* and *b* (µg/mL) extracted using acetone

Acetone (99.5%)			
Microalgae 1	(µg/mL)	Microalgae 2	(µg/mL)
Chlorophyll *a*	3.39	Chlorophyll *a*	3.30
Chlorophyll *b*	2.80	Chlorophyll *b*	2.95
Total chlorophylls	6.196	Total chlorophylls	6.255

Table 1 shows the result calculated by using composed equations for chlorophyll a, chlorophyll b and total chlorophylls. Concentration of chlorophyll *a* and *b* were almost the same as expected for both samples extracted using acetone as the solvent with 3.39 µg/mL and 3.30 µg/mL for chlorophyll *a* for microalgae 1 and 2 respectively. The amount of chlorophyll *b*, are 2.80 µg/mL for microalgae 1 and 2.95 µg/mL for microalgae 2. Total chlorophylls for both samples not shows significant differences with 6.196 µg/mL for microalgae 1 and 6.255 µg/mL for microalgae 2. Acetone 99.5% give similar effect for both microalgae .

Table 2: The concentration of chlorophyll *a* and *b* (µg/mL) extracted using methanol

Methanol (99.5%)			
Microalgae 1	(µg/mL)	Microalgae 2	(µg/mL)
Chlorophyll *a*	2.52	Chlorophyll *a*	2.36
Chlorophyll *b*	1.15	Chlorophyll *b*	1.32

Methanol (99.5%)			
Total chlorophylls	3.66	Total chlorophylls	3.68

Based on the Table 2, which shows the concentration calculated by equations and Beer' law, for methanol as the extraction solvent, the concentration of chlorophyll *b* is quite lower than chlorophyll *a*, with 1.15 µg/mL for microalgae 1 and 1.32 µg/mL for microalgae 2. The amount of chlorophyll *b* from microalgae 2 was higher than microalgae 1 in contrast with chlorophyll *a*. However the total amount of chlorophylls are almost similar for both microalgae.

Table 3: The concentration of chlorophyll *a* and *b* (µg/mL) extracted using ethanol

Ethanol (95%)			
Microalgae 1	(µg/mL)	Microalgae 2	(µg/mL)
Chlorophyll a	2.45	Chlorophyll a	2.34
Chlorophyll b	1.50	Chlorophyll b	1.30
Total chlorophylls	3.98	Total chlorophylls	3.71

Finally, Table 3, shows the concentration the concentration of chlorophyll *b* is quite lower than chlorophyll *a* for both microalgae calculated by equations and Beer' law and directly affected the amount of total chlorophylls after using ethanol as the extraction solvent.

As reported earlier, pigmentation microalgae shows a great diversity and different groups of algae have different pigment composition as well. As pigments usually can be found in chromophores which is a specialized plastids, all major algal groups will have minimum one pigment in their cells. Chlorophylls are the main pigments and being responsible for the green colour of the algae. As a chlorine pigment, it is related to porphyrin which contains iron compound (heme). Its chemical structure has the magnesium (Mg^{2+}) in the centre of the ring.

Of the three extraction solvents tested, for both samples, the best solvent for the extraction of chlorophyll *a* and chlorophyll *b* is acetone (99.5%). As a standard solvent for the chlorophylls extraction, it has given better result than the other two solvents because of its high in solubility among the three solvents. One-way Anova was carried out for all type of solvents. When comparing effect of solvent, it was determined that p-value were no significant difference in the yield for three solvents. Research has shown that the type of solvent significantly affects the extraction efficiency since it was determined that $F>F_{crit}$ when comparing the effect of solvent on extraction efficiency (Chan, 2012). In that research, an Anova test for two factors with replication was carried out. But in this research, one-way Anova with single factor was tested since there is no other factors apart from type of solvent. Results from this study in contrast with Simon and Helliwell, Sartory and Grobbelaar and Jeffrey et al. that found methanol and ethanol to be superior extraction solvents to acetone.

However, the data obtained important as the guide to optimize the pigment extraction process from locally isolated microalgae. The amount of chlorophyll extracted from a particular algal species was found to be highly dependent on its growth stage. Information about microalgae extracted in the stationary growth phase were shown to have significantly higher amount of chlorophyll *a* compared to the same species obtained in the logarithmic phase[35] was used as the guidance to choose the growth stage of the microalgae for pigment extraction.

Conclusion

The results from this study clearly concludes that extraction of pigments from microalgae by different extraction solvents are depends on the chemical nature of the pigments that present in the algae. Medium, light intensity, temperature and aeration will influence the growth of the microalgae. Different pigments require the effective solvent to give better result. This preliminary study indicated that the locally isolated microalgae have potential to produce pigment. Acetone (99.5%) is the best solvent for the extraction of chlorophyll a and chlorophyll b from microalgae isolated from Selangor sea water. Data obtained

important as the guide to optimize the pigment extraction process from locally isolated microalgae.

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