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## Growth and Lipid Compositions of Locally Isolated Microalgae

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### ABSTRACT

Nine different strains of microalgae were found in samples of local freshwater. One strain in particular which is referred to as BA strain due to its similar characteristics to *Botrydiopsis arrhiza* was found to show potential in lipid production as its biomass accumulation and lipid yield were comparable to that of the commercial strain *Chlorella fusca*. This study identifies the potential of local algae based on its lipid compositions as indication for possible biofuel feedstock.

**Key words:** Local microalgae, biofuel feedstock, *Botrydiopsis arrhiza*, *Chlorella fusca*

### INTRODUCTION

In recent years, the potential prospect of microalgae for sustainable energy development have been extensively reviewed and microalgae are foreseen to be the fuel of the future. In fact, microalgae biofuels have been placed globally as one of the leading research fields which can bring enormous benefits to human beings and the environment (Lam and Lee, 2012). The use of microalgae as bio-energy feedstock seems to be promising because their biomass doubling times in microalgae during exponential growth are commonly as short as 3.5 h and their lipid content could be adjusted through altering growth media composition. Due to their simple cellular structure, microalgae have higher rates of biomass per oil production than conventional crops. Oil content in microalgae can exceed 80% by weight of dry biomass. Therefore microalgae can produce 30-100 times more energy per hectare as compared to terrestrial crops. Beside that microalgae can be harvested batch-wise nearly all year-round providing a reliable and continuous supply of oil. Furthermore, microalgae use carbon dioxide in the atmosphere as a source of carbon for growth and can also be cultured in salty or waste water (Phukan *et al.*, 2011).

Through the process of photosynthesis, algae convert water, carbon dioxide and light into oxygen and biomass. At the end of the process, according to the characteristics of the micro-algal biomass obtained, it can be used to produce biodiesel, ethanol, hydrogen, biogas or direct burning and are not precursors of problems caused by fossil fuels and

renewable energies (Costa and de Morais, 2011; Amin, 2009). Nowadays, there are two kinds of cultivation systems: Open pond and closed photo-bioreactors system. Closed photo-bioreactors system has more efficiency but it cannot be applied in industry because of its high cost. Until now, transesterification is the common way to produce biodiesel from the oil of algae. The transesterification reaction uses super critical fluids, enzymatic, acid-catalysed or alkali-catalysed (Costa and de Morais, 2011; Fukuda *et al.*, 2001).

Zhou *et al.* (2011) collected sixty algae-like microorganisms from different sampling sites in Minnesota to select high-lipid producing facultative heterotrophic microalgae strains capable of growing on Concentrated Municipal Wastewater (CMW) for simultaneous energy crop production and waste water treatment. Among twenty-seven strains found, five strains have the ability to adapt to CMW, high growth rates ( $0.455-0.498 \text{ day}^{-1}$ ) and higher lipid productivities ( $74.5-77.8 \text{ mg L}^{-1} \text{ day}^{-1}$ ) (Zhou *et al.*, 2011). Krohn *et al.* (2011) also found out wild algae which were isolated from Minnesota lakes and ponds have high productivity and sufficient lipids for further use in biodiesel production studies when comparing with *D. tertiolecta* (Krohn *et al.*, 2011). Algae are a photosynthesis organism, where concentration of  $\text{CO}_2$  supplied during growth is very important. There are many studies that associate the importance of identifying the  $\text{CO}_2$  concentration with growth. With *Chlorella* sp. the suitable range of  $\text{CO}_2$  concentration is 0.33-15% (Suali and Sarbatly, 2012).

Most of the algae in past studies with regards to bio-fuel of commercial algae focus more on their potential. Some studies on locally isolated algae found that local algae also have the potential to produce lipid as a feedstock for bio-fuel but studies on local algae in Malaysia are rare. Cell disruption technique is the important step to extract the lipid content. Ultrasonic and microwave are two common methods for this step. Based on Lee *et al.* (2010) the microwave oven method was identified as the most simple, easy and effective for lipid extraction from microalgae.

Algae used in this study were extracted from various freshwater locations in Malaysia such as river and pond. The algae are first classified for their characteristics prior to lipid extraction studies.

## MATERIALS AND METHODS

The methodology is divided into sampling, cultivation, strain isolation, growth and lipid composition analysis. Firstly, samples were collected from the freshwater locations which are river and lake. The collected samples were then transported to the laboratory for cultivation and isolation. After a pure microalgae strain was obtained, the process continues to the growth analysis step based on the absorbance, cell concentration and biomass accumulation. Finally, the lipid was extracted from the microalgae by using different solvent and analyzed using GC-MS.

**Cell concentration:** To obtain the cell concentration, 5 mL of sample culture was extracted from the cultivation tank using micro pipette. The sample culture was left to settle for 15 min. The mirror-like polished surface and cover slips were cleaned with lens paper and ethanol. The cover slip was placed over the counting surface. The cell suspension was introduced into one of the V-shaped wells using Pasteur pipette to allow the area under the cover slip to fill by capillary action. The counting chamber was placed on the microscope stage and the counting grid was brought into focus at low power.

**Absorbance:** Absorbance is the rapid way to measure the growth of microalgae. The wavelength range usually used to measure is from 550-750 nm as the absorption of chlorophyll and some photosynthesis pigments is at a minimum to avoid the interference by chlorophyll and these pigments. The 3 mL of sample culture from each tank was extracted using micro pipette and the culture was placed in a quartz cuvette. The OD of the culture was measured at the wave length in the visible range (400-700 nm). Each sample was diluted to give an absorbance in the range of 0.1-10 if the optical density is greater than 1.0. After the primary measurement, the OD was calculated based on the chlorophyll in the culture. The chlorophyll is one of the pigments contained in microalgae and measured at 688 nm by the UV-Vis spectroscopy.

**Lipid composition analysis:** The lipid composition analysis uses the Bligh and Dyer (1959) method and was conducted

every 2 days since day 1 of growth. After harvesting, the biomass was dried and the cell was disrupted by microwave or ultrasonic. Then the biomass is extracted via soxhlet apparatus. The analysis of lipid composition was done using GC-MS.

## RESULTS AND DISCUSSION

**Cell concentration:** Based on the growth curves in Fig. 1, the lag and acceleration phases of BA were not clearly defined compared to the CF strain. Based on the graph; this condition takes place within a few hours of cultivation. The exact time was not recorded as the results were taken at 24 h intervals. Therefore, the trend of the lag and acceleration phases of BA was not able to be observed. This also means that BA strain is has a faster growing rate at the beginning of growth compared with the CF strain. After day 8, the BA and CF strain almost reached a stationary phase at the same time although at different concentration. This may have been affected due to the suitability of the CF and BA strain with the culture medium. When the culture condition was changed in terms of nutrient content and light intensity, the growth of microalgae reached retardation phase and then come to a stationary phase.

**Absorbance:** Figure 2 shows the OD of CF and BA culture with solely air. BA strain has higher OD than CF strain and both reach the stationary phase at day 8. These results are similar with the results of cell counting. There is one exception that the lag phase of BA strain is quite clear in this result while the cell counting results is reverse. This may be due to the condition at the beginning where the number of cells was minimal and the cells were adapting to the new environment. Maybe due to that, the pigments in the cells were not apparent to the naked eye and thus the absorbance reading was 0. However, when observing though the microscope, the cells were visible and can still be counted.

**Lipid compositions:** Based on the data shown in Table 1, the lipid compositions under different extraction methods showed

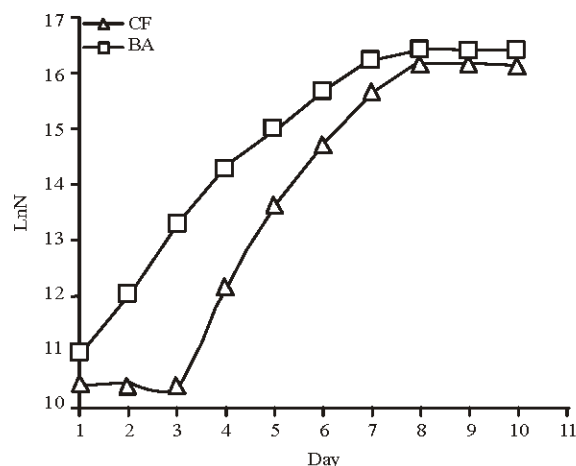


Fig. 1: Growth curve of CF and BA

Table 1: Lipid compositions and percentage of relative mass (relative formula mass of the substance)

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Composition	Extraction method						
	Bligh and Dyer (1959)			Soxhlet extraction (CHCl <sub>3</sub> )			
	CHCl <sub>3</sub>		MeOH	Microwave		Ultrasonic	
	CF	BA		CF	BA	CF	BA
C12	21.29						
C13					3.60		14.92
C14	5.11		3.63		6.30	4.30	0.04
C15				5.43		1.85	5.32
C16	1.05			39.70		19.97	1.78
C17					12.45		
C18			4.75				0.69
C19						32.54	1.89
C20					2.67		5.13
C21	6.51				2.39		
C22				5.35		1.24	

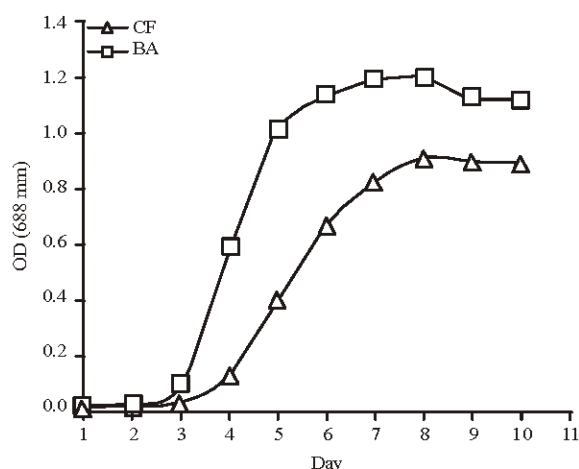


Fig. 2: Absorbance of CF and BA

different concentrations. Although mixture of CHCl<sub>3</sub>: MeOH was known as the best solvent for lipid extraction, the percentage of relative mass of its compositions was lower than Soxhlet extraction with CHCl<sub>3</sub> as a solvent extraction. It may be due to the time taken for the Bligh and Dyer (1959) method was shorter than Soxhlet extraction and it was not supported by cell disruption. Between the microwave and ultrasonic, the lipid compositions extracted using ultrasonic as a cell disruption technique show more variety and higher relative percentage than the microwave. Therefore, ultrasonic is a better cell disruption method than the microwave and CHCl<sub>3</sub> is a suitable solvent to extract the lipid content of BA and CF with Soxhlet extraction.

The percentage relative mass of BA compositions were slightly lower than CF strain but the lipid compositions of BA strain was more varied than CF strain under the respective methods. This shows that the BA strain still has the potential in lipid production as its various compositions easily suited to biodiesel compositions. With regards to the percentage of relative mass, BA strain has the highest relative mass at 12.5 and 14.9% with microwave and ultrasonic as a cell

disruption which is higher compared with the Bligh and Dyer (1959) method. Therefore, the lipid content of BA can be extracted more with another method of extraction. Besides that, the cultivation conditions are also an important component that may influence the lipid compositions. This means that if the BA strain is cultivated under suitable conditions, it will produce more lipid content which yield and compositions are more suitable.

## CONCLUSION

The growth curves of CF and BA are almost the same. It means that, the BA strain or the locally isolated algae are suitable to be cultivated in BBM medium and grow as well as the commercial strain does. The composition of lipid content is different as it depends on the solvent extraction as well as the cell disruption. Based on the results, the local algae have various carbon numbers; it means that local algae have the potential to be used for lipid production.

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