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# ISOLATION AND CHARACTERIZATION OF POTENTIAL TROPICAL OLEAGINOUS MICROALGAE FOR BIOFUEL PRODUCTION

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**SUMMARY:** Recent volatility in crude oil prices attributed to increase demand and limited resources, tied with the urge to reduce pollutant emissions and greenhouse gases, have created a major focus in the production of sustainable biofuel. As one of the promising feedstocks, microalgae appeared to be a new source of renewable biofuel that is capable of meeting the global demand for transport fuel. Four green microalgae morphologically identified as *Staurastrum* sp., *Scenedesmus* sp., *Desmodesmus* sp., and *Ankistrodesmus* sp. were isolated from Kuala Selangor Nature Park and Hulu Langat River, Selangor. The intracellular lipid bodies of the microalgae were stained with BODIPY 493/503 for the screening of the potential microalgae for biofuel production. From the staining, more lipid bodies can be seen from *Scenedesmus* sp. compared to the other three isolated microalgae species.

***Keywords*—** Biofuel, BODIPY, isolation, lipid, microalgae

## INTRODUCTION

Over the past two decades, countries around the world have been progressively developing their industrial sector. This rapid development of industries has tagged along the increasing needs of energy [1]. While the global demand of energy is continuously ascending, the basic source of energy is remarkably depleting. This would bring a huge problem to the entire world.

At present, microalgae had become the promising alternative and being considered as the potential feedstock for the production of third generation of biofuels [2]. This is due to the reasons that biofuel derived from microalgae gives some merits than those other alternative feedstocks. Microalgae have the potential of giving twenty times more productivity in terms of oil than oilseed crops [3]. Microalgae also grow faster and this high growth rate results in high biomass yield in a short period of time [4]. Lipid bodies in microalgae are accumulated specifically under the conditions of excess carbon and limited of other nutrients, especially nitrogen [5]. The accumulated lipid is in the form of discrete oil droplets and in some cases it can occupy up to 85% of the cell volume [6].

The objectives of this study were focused on the isolation of the tropical oleaginous microalgae and the comparison of their lipid content for the potential production of biofuel.

## 2. MATERIALAS ANDMETHODS

### 2.1 Isolation and inoculation

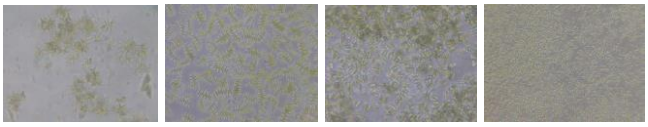
Microalgae samples were collected from freshwater in Kuala Selangor Nature Park and Hulu Langat River with a plankton net (25µm open mesh). Four individual cells of microalgae were isolated from the water samples using sterile micropipette washing method and inoculated in 96 well plates containing artificial freshwater (AF-6) medium at ambient temperature under continuous illumination of 45 µmol photons $m^{-2}s^{-1}$ light intensity. The isolated microalgae were morphologically identified as *Staurastrum* sp., *Scenedesmus* sp., *Desmodesmus* sp., and*Ankistrodesmus* sp.by referring to Encyclopaedia of Microbes in Freshwater: Visual Guidebook of Protozoa as shown in Fig 1.

### 2.2 Algal cultures

The isolates were cultured in 100 mL Erlenmeyer flasks containing 75 mL of AF-6 medium at ambient temperature under continuous illumination of 45 µmol photons $m^{-2}s^{-1}$ light intensity. The optical densities (OD) of each sample are determined using UV spectrophotometer at 750nm by withdrawing aliquots of the microalgae cultures at every 24 hours. All experiments were carried out at least in duplicate.

### 2.3 Fluorescent staining

One type of the available lipophilic dyes which is BODIPY 493/503 (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) was prepared in dimethyl sulfoxide (DMSO) to give a stock solution of 1 mg $mL^{-1}$ and stored in dark tube protected from light. 1 mL of microalgae cultures were stained with 1 µL of BODIPY 493/503 followed by incubation in darkness for 10 minutes before being observed under the fluorescence microscope [7].



(a) (b) (c) (d)

Fig 1. Visualization of isolated cultures of microalgae at 20X magnification. (a) *Staurastrum* sp. (b) *Scenedesmus* sp. (c) *Desmodesmus* sp. (d) *Ankistrodesmus* sp.

## 3. RESULTS & DISCUSSIONS

### 3.1 Growth analysis

Fig. 2 shows the comparison of growth curve among four isolated microalgae based on the optical density analysis by UV spectrophotometer at 750 nm. From the curve, *Ankistrodesmus* sp. showed the fastest growth followed by *Desmodesmus* sp., *Staurastrum* sp. and the slowest one was the *Scenedesmus* sp. After 8 days cultivation, the microalgal growth was still at the exponential phase. This means that there were still enough nutrients in the medium for the growth of the microalgae. Upon reaching the stationary phase where most of the essential nutrients were deprived, the microalgae stop their cell division and start to accumulate lipid in their cell bodies [2].

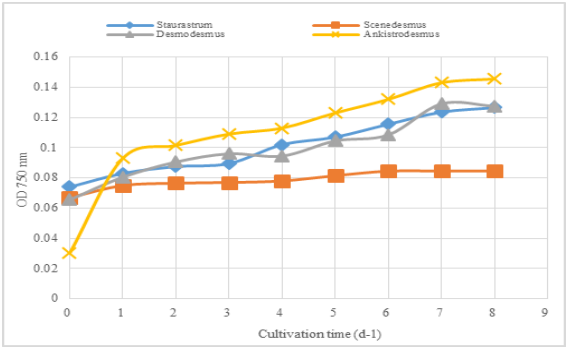



Fig 2. Growth curve of four isolated microalgae *Staurastrum* sp., *Scenedesmus* sp., *Desmodesmus* sp., and *Ankistrodesmus* sp.

### 3.2 Fluorescent staining

The isolated cultures were stained with BODIPY 493/503 and being observed under fluorescent microscope. Staining the microalgae with BODIPY 493/503 aids in the detection of intracellular lipid bodies by fluorescence microscopy [7]. This is because the fluorescence intensities could be correlated to the lipid content within the microalgae cells. The BODIPY 493/503 stained lipid bodies showed bright green fluorescence in the microalgae cells as shown in Fig. 3. Meanwhile, the strong red fluorescence were from the chlorophyll of the microalgae.

From Fig. 3, it can be deduced that *Scenedesmus* sp. contained more lipid bodies compared to the other isolated microalgae. Strong green fluorescence separated from the red fluorescence stained by the BODIPY 493/503 can be seen clearly in *Scenedesmus* sp. cells. From the BODIPY 493/503 staining, not much lipid bodies contained in *Staurastrum* sp., *Desmodesmus* sp., and *Ankistrodesmus* sp. cells as only some green lipid droplets can be seen.

(a) (b) (c) (d)

Fig 3. Visualization of intracellular lipid bodies of four isolated microalgae cells under normal light and fluoresecent light at 40X magnification when staining with BODIPY 493/503.
(a) *Staurastrum* sp. (b) *Scenedesmus* sp. (c) *Desmodesmus* sp.
(d) *Ankistrodesmus* sp.

More lipid bodies are expected to be seen when staining the microalgae cells after the cultures reached the stationary phase of growth as the lipid accumulation is increased under the nutrient starvation condition. However, gravimetric analysis involving transesterification process and gas chromatography are needed to study and characterize the composition of the lipid content of the isolated microalgae for the determination of potential biofuel production.

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