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مروري مقاله

آزاده لاسرسی

په دوه لښته فوتو بایوریکتور کې د کلوریلا نوعې (*Chlorella* sp) کښت

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لنډیز

له فوسیل څخه لاسته راغلي سونګ توکي په نړیواله کچه د بېلابېلو موخو لپاره د انرژیکي سرچینو په توګه کارول کېږي. په هر صورت له فوسیل څخه لاسته راغلي سونګ توکي د دې په خاطر چې نه نوی کېدودونکی خاصیت لري له همدې املې په تدریجی ډول بایوفویل ته اړول شوي دي. ددې ژوندیو موجوداتو د مختلفو نسلونو په منځ کې د مایکرو الجې له درېیم نسل څخه کار اخیستل شوی دی چې د سوداګریزو صنعتونو لپاره غوره ګڼل کېږي. د مایکرو الجي د درېیم نسل په واسطه د انرژي تولید یو اغیزمن انتخاب دی ځکه چې دا د نباتي محصولاتو په شرایطو کې ستونزه نه رامنځته کوي. د کلوریلا په نامه د مایکرو الجي یوه نوعه چې له تازه اوبو څخه را ایستل شوې ده، د مایکرو الجي کښت یا په لابراتوارونو کې مسلکي کرنه د غیر مشبوع شحمي تیزابونو (PUFAs) په تولید کې خورا مهم رول لوبوي، په ځانګړې توګه شحمي تیزابونو لکه میتایل ایستر (FAME) چې د درېیم نسل د بایوفویل په تولید کې کلیدی جز ګڼل کېږي. سربېره پردې چې کلوریلا نوعه د دې وړتیا هم لري، چې تر لوړ فشار لاندې وکړل شي لکه د هوا لوړه کچه، pH او د انوکولم اندازه کیداې شي چې ډېر شحمي تیزابونه راټول او تولید کړي. له همدې املې د دې څېړنې تمرکز له تازه اوبو څخه استخراج شوې کلوریلا نوعې الجي باندې دی چې په مورفولوژیکي ډول پېژندل شوی او د هغه د ودې په پروفایل باندې هم په لنډه توګه نظر اچول شوی دی، د کلوریلا نوعې ډله ییز کښت به د درېیم نسل بایوفویل په تولید باندې د پام وړ اغېزه ولري، کوم چې د پایښت لرونکي انرژۍ یوه نوې سرچینه ده.

کلیدي کلمې: کښت، استخراج کول، کلوریلا نوعه او فوتوبایوریکتور.

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دغه ژورنال د وردک پوهنتون په چوکاټ کې د لوړو زدکړو وزارت د رسمي جواز پر اساس فعالیت کوي.

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Cultivation of *Chlorella* sp. in 2 liter Photobioreactor

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Abstract

Fossil fuels have been used globally as one source of energy for different types of application. However, the dependency towards fossil fuels have been shifted gradually to biofuel due to its non- renewable characteristic. Among the biofuelgenerations, third-generation biofuels which are derived from microalgae have been preferred by commercial industries. Third generation biofuel is a promising option because it does not have issues in term of crops supply. *Chlorella* sp. is one of the freshwater microalgae that are being mass cultivated due to its unique characteristic in polyunsaturated fatty acids (PUFAs) production, particularly fatty acid methyl ester(FAME) which is the key element in third generation biofuel production. Furthermore, *Chlorella* sp. are able to accumulate more fatty acid when cultivated under stresscondition such as high aeration rate, pH and inoculum size. Hence, the focus of thestudy would be on the morphological identification, the growth profile of freshwaterisolated *Chlorella* sp. In a nutshell, the mass cultivation of *Chlorella* sp. would have significant effect on the production of thirdgeneration biofuels which is a new source of sustainable energy.

Keywords: cultivation, isolation, Chlorella sp. and photobioreactor

INTRODUCTION

Oil and fossil fuels are the essential of modern economies for the past few centuries tillnow, its applications include supporting human mobilities, cosmetic industries and generating electricity. However, fossil fuel is not a source of renewable energy. The use of liquid fossil fuel as an energy source has long been considered unsustainable and most importantly the liquid fossil fuel will be diminished by the middle of this century (Alam *et al.*, 2015). According to the recent report review of world energy 2019, there are 99843 thousand barrels of fossil fuels being consumed by the world daily, while there are only 94718 thousand barrels of oil being produced.

The unsustainability and continuous exhausting of non-renewable fuel sources have initiated an interest in the effort of looking for new source of renewable energy with low emission of greenhouse-gas performance which is the biofuel (Chiappe *et al*., 2016). Generally, there are few generations of biofuel, and the emergence of the later generation of biofuels are to improveand to solve the problems encountered such as costing for pre-treatment.

Microalgae are microscopic organisms that live in fresh and marine waters and able to carry out photosynthesis to produce their own food (Suganya *et al.*, 2016). *Chlorella* sp. is representing the Chlorophyceae class. It is unicellular gree algae, spherical, and its reproduction is a sexual. It is an autotrophic and containing the photosynthetic pigments which located in its chloroplasts. *Chlorella* sp. could be found mainly in freshwater and soils. Moreover, they have high capacity for photosynthesis, which able to reproduce in several hours and requiring only sunlight, carbon dioxide, water and a small amount of nutrients (Silva *et al.*, 2019). The genus *Chlorella* sp. consists of small, spherical to ovoid, nonmotile, unicellular or colonial microalgae with a single chloroplast with a pyrenoid (Bock *et al.*, 2011). In addition, *Chlorella* sp. Cells are autospores which reproduce a sexual mitosis. *Chlorella* sp. have high levels of chlorophyll when compared to several other species of microalgae (de Morais *et al.*,2015). Apart from that, according to (Abinandan & Shanthakumar, 2015), microalgae show a substantial part in meeting the energy demand and also function as the most important feedstock for sustainable products. Microalgal biomass could be used for biodiesel, feed, and food production (Duong *et al.*, 2015). One of the main advantages of using microalgae in biofuel production is that it does not compete with other crops in land use, for example during the production of first-generation biofuel which require large quantity of crops as the raw materials (Adeniyi *et al.*, 2018). Among the microalgae species, *Chlorella* sp. have been chosen and widely used in biofuel production due to its characteristic which is able to grow continuously either in stable or fluctuating

environment, easy to harvest and its significant high and constant extractable lipid content (Shuba & Kifle, 2018). In addition, Physicochemical stresses such as UV-treatment, temperature, pH, salinity and nitrogen-deficient could be used to improve the lipid productivity in microalgae (Chi *et al*., 2019). Hence, cultivation of *chlorella* sp. has become an interesting topic to investigate.

Problem statement

In the recent years, mass cultivation of microalgae has become an interest for all the researchers and investors due to its great benefits with the potential in which able to overcome the global energy crisis (Shuba & Kifle, 2018). Despite of that, open cultivation system has always been the most preferred choice among other cultivationsystems as it could reduce the capital cost of mass cultivation of microalgae commercialisation purposes (Jerney & Spilling, 2018). However, there are challenges needed to overcome by implementing the open cultivation systems, environmental factors such as rainfall, solar radiation and biological factors such as light, pH and salinity have become parameter that affect the biomass productivity in the open pond system (Kumar *et al.*, 2015). Photobioreactor have been proposed as it is designed to increase the photosynthetic efficiency, higher biomass concentrations, lower risk of contamination, prevent loss of water through evaporation under a controlled environment (Margarita V Rodionova *et al.*, 2017). In addition, various research indicates that to endure adverse environmental conditions, microalgae typically store lipids in the form of triglycerides. These lipid content can be further enhanced in microalgae by manoeuvring the cultures and subjecting them to diverse stress conditions (Chi *et al.*, 2019). Hence, mass cultivation of microalgae with photobioreactor would likely to have a better result as the microalgae are being cultured in a controlled environment which could maximize and enhance the qualityof produced lipid which needed as biofuel feedstocks.

Research Objectives ,The objectives of the research are:

(a) To screen, isolate and identify *Chlorella* sp. from local environment.
(b) (b) To study the growth performance of locally isolated microalgae *Chlorella* sp. And coal-fired power plant isolated *Chlorella sorokiniana*.

Scope of the study

The scope of this study is to isolate *Chlorella* sp. from the freshwater sample collected from the lake in Ayer Hitam, Johor. Isolation steps such as serial dilution, pour plate, spread plate and streak plate were performed to isolate the microalgae that hasthe similarmorphology with *Chlorella* sp. After isolation and identify the *Chlorella* sp morphologically, it was cultivated in the conical flask to study its growth profile. At the same time, the growth

profile of the marine isolated *Chlorella sorokiniana* was studied as well to determine the best growth performance strain.

Significance of the study

Malaysia is heavily dependent on fossil fuel and natural gases especially in the industrial and transportation sector which are one of the major source of pollution (Mushtaq *et al.*, 2013). Fossil fuel and natural gases are non-renewable energy and with the emerge of biofuel as a new source of renewable energy could lighten the demand of fossil fuels. Unsustainability and continuous exhausting of non-renewable fossil fuels have initiated looking for renewable fuel sources to fulfil the world demand (Mathimani & Pugazhendhi, 2019). Malaysia palm based biofuel have great potential to become producer of renewable energy besides producing oil to feed the world (Loh & Choo, 2013). On the other hand, Malaysia is producing variable wastes from both agro-industrial and industrial sectors which comprises of high nitrogen and phosphorus could be recycled as the nutrient supply for microalgae (Jayakumar *et al.*, 2017). Furthermore, optimising the growth condition of *Chlorella* sp. in low cost 2 litre photobioreactors would reduce the difficulties in mass production of microalgae at the same time reduce the risk of contamination. Next, through the study on the lipid profile would likely to initiate the mass cultivation of locally isolated *Chlorella* sp. As an alternative way for biofuels production in Malaysia.

RESEARCH METHODOLOGY

Operational framework

Generally, the experiment started with the isolation and morphological identification of *Chlorella* sp. in the collected freshwater sample. After morphological identification, the growth profile of freshwater *Chlorella* sp. and *Chlorella sorokiniana* isolated from the coal-fired power plant were obtained by cultured them in the conical flasks. Then, the growth performance of both *Chlorella* sp. strains was compared, and the strain with the best performance were then cultured in the 2L photobioreactors. size.

In addition, the isolation and morphological identification of freshwater isolated *Chlorella* sp. include performing serial dilution of the collected water sample at the water pond in Ayer Hitam, Johor. After that, the diluted freshwater sample were cultured in the BG 11 agar plate and observed under the microscope (DM750) with 100x magnification to identify the potential *Chlorella* sp. based on the morphological characteristics. Apart from that, the culturing technique that were used are fermentation technique which include optimising the 2 Litre photobioreactor where the *Chlorella* sp. were cultured.

Collection of microalgae

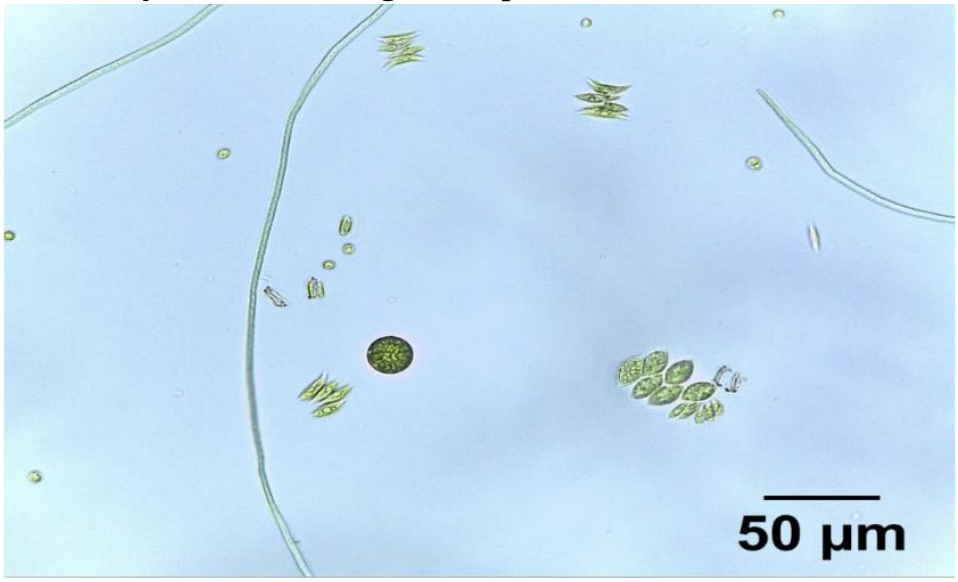
The freshwater samples were collected from the lakeside at Ayer Hitam, Johor, Malaysia. The sample of freshwater were collected at 3 different depth using sterile universal bottles. Next, the freshwater sample were viewed under the light microscope Leica DM750 at 10X magnification to determine the presence of any potential species of Chlorella. After observation, the freshwater samples were stored in the refrigerator at 4°C to preserve the microalgae species in the collected water samples.

Isolation of pure culture

Isolation of pure culture were done using the serial dilution technique, spread plate and streak plate. First, the unsterile and mixed population water sample which collected were viewed under the microscope Leica DM750 to identify whether there is any potential species that have the similar morphology with *Chlorella* sp. which is rounded in shape and green in colour. After identification, the water sample were diluted using serial dilution technique before spread on top of the BG11 agar plate. Next, the agar plate spread with the water sample were placed under white light with 3000 Lux at room temperature for the microalgae to grow. After that, the colonies grow were harvest and viewed under the microscope Leica DM750 before streaking on the BG 11 agar plate to obtain the pure culture. The selected colonies were transferred into amicrocentrifuge tube filled with distilled water and mix using avortex to ensure the cells were homogenized.

RESULTS AND DISCUSSION

Observation of microalgae species from freshwater sample The freshwater sample collected from the lakeside at Ayer Hitam, Johor was cultivated in the 250 mL conical flask with 150 mL working volume containing BG 11 media for 14 days. The microalgae sample in the conical flasks were observed using



microscope LeicaDM750 and there are mixed population in the culture. Figure 1. shows the morphology of the mixed population observed using the microscope Leica DM750 with 100x magnification.

Figure 1. The morphology of the mixed population observed using the microscope Leica DM750 with 100x magnification.

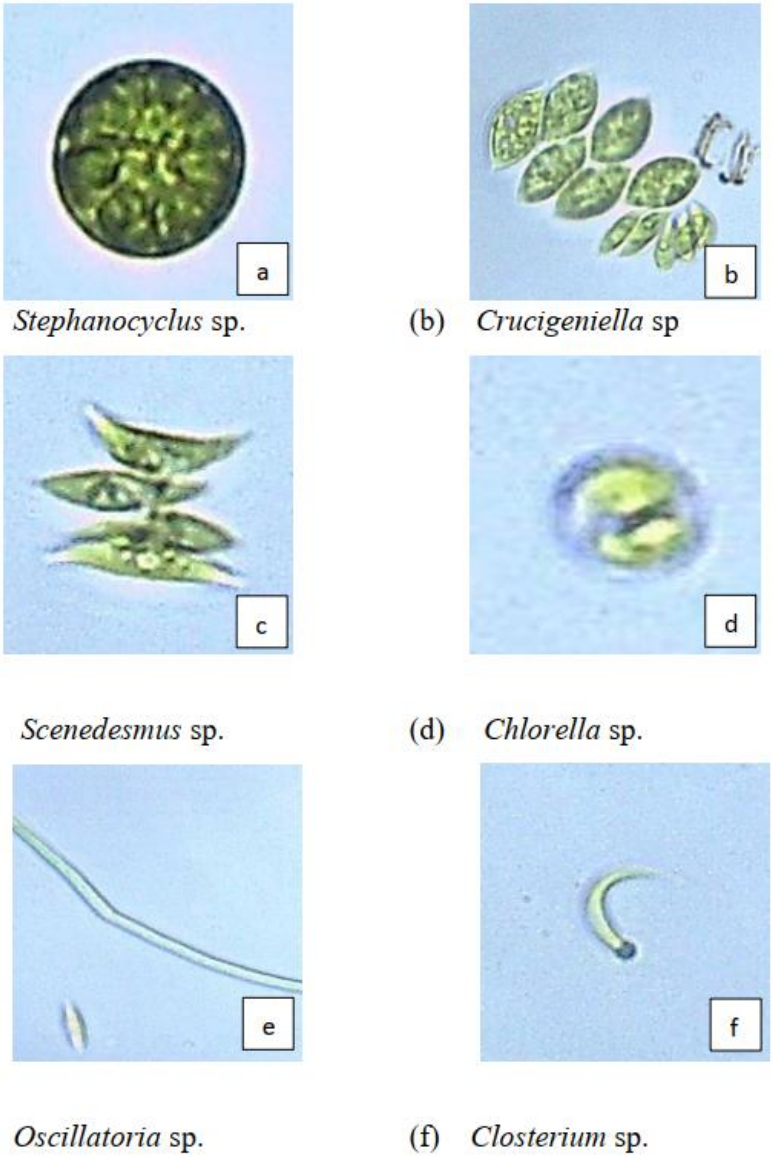
From Figure 1.1, the microbes were found green in colour which indicated that the microbes containing the photosynthetic pigments such as chlorophyll and mixture of carotenoids such as astaxanthin, B-carotene, canthaxanthin and echinenone (D. P. Singh *et al.* 2019). Furthermore, based on the different morphology of microorganisms found in the water sample, it might contain the *Chlorella* sp., *Scenedesmus* sp., *Oscillatoria* sp., *Crucigeniella* sp., *Closterium* sp. And *Stephanocyclus* sp (Sekimoto et al., 2012; Thakar et al., 2018). Figure 2 (a

- f) shows the different morphology of the microalgae with the respective possible genus.

Figure 2 (a-f) The different morphology of the microalgae with respective possible genus.

Isolation of potential *Chlorella* sp. from the collected freshwater sample

The isolated microalgae were cultivated with 3000 Lux white light in the 250 mL conical flask with 150 mL of BG 11 medium for 7 days at 10 % inoculum



(a) *Stephanocyclus* sp. (b) *Crucigeniella* sp

(c) *Scenedesmus* sp. (d) *Chlorella* sp.

(e) *Oscillatoria* sp. (f) *Closterium* sp.

size to enrich the potential *Chlorella* sp. population in the water sample before spreading on the agar plate. After the reshwater sample collected was cultured for 9 days, the culture was suspected to be dominated by other microalgae species as there was a significant colour change from transparent green colour to dark green colour. Consequently, the dark green colour freshwater sample were then observed under the microscope Leica DM750 with 400x magnification and the results show that the other species with different

morphology have become dominant species in the freshwater sample. Figure 3 (a) to Figure 3 (c) shows the dominant species in the freshwater sample. Based on the figures, the dominant species could be *Crucigeniella* sp, *Oscillatoria* sp.,*Scenedesmus* sp. and *Closterium* sp. according to the respective morphology *(*Kim *et al.*, 2014*)*.

Figure 3 (a). Microalgae which could be Scenedesmus sp. is dominant in the freshwater sample.
Figure 3 (b). Microalgae which might be *Closterium* sp. is dominant in the freshwater sample.

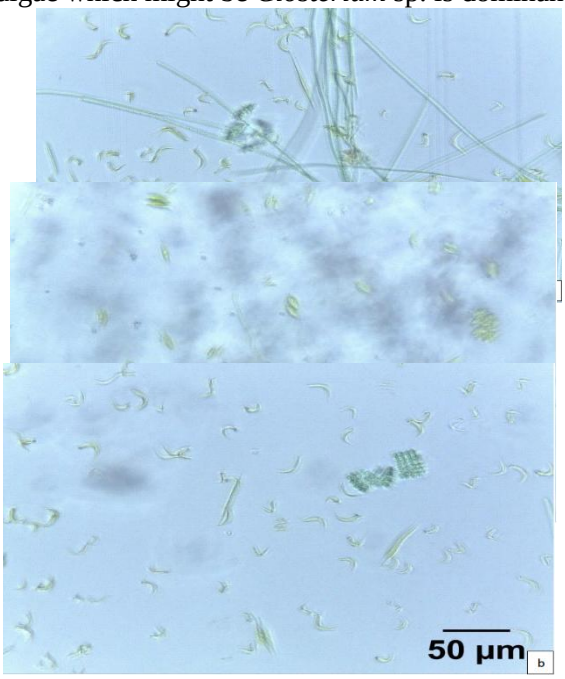


Figure 3 (c). Microalgae which is believed to be *Closterium* sp. and *Oscillatoria* sp. is the dominant species in the freshwater sample.

The potential *Chlorella* sp. that was found from the freshwater sample was not the dominant species. After investigation, there were presence of microzooplanktonic grazers in the dark green colour freshwater sample and that was the reason the potential *Chlorella* sp. was not dominant in the freshwater sample.

Figure 3 (d) below shows the presence of microzooplanktonic grazers in the freshwater sample viewed using microscope Leica DM750 with 10 times magnification, while Figure 3 (e) and Figure 3 (f) shows the close-up image of the microzooplanktonic grazers at 100x magnification.

According to the research conducted by (Day *et al.*, 2017), these microzooplanktonic grazers are a singular grazers that exhibit preference for

certain food and the size of its prey as well as the chemical characteristic of the prey have a significant influence on its grazing preferences.

Hence, the potential *Chlorella* sp. That was found in the freshwater sample might be consumed by these microzooplanktonic grazers in the same freshwater sample because the size of *Chlorella* sp. is small with 2-10µm diameter with high nutritional value (Safi *et al.*, 2014). In contrast, there were other microalgae species become dominant in the freshwater sample, the main reason of that is the dominant microalgae species are less susceptible to be ingested by these zooplanktonic grazers due to their physical characteristics which is larger in size (Day *et al.*, 2017).

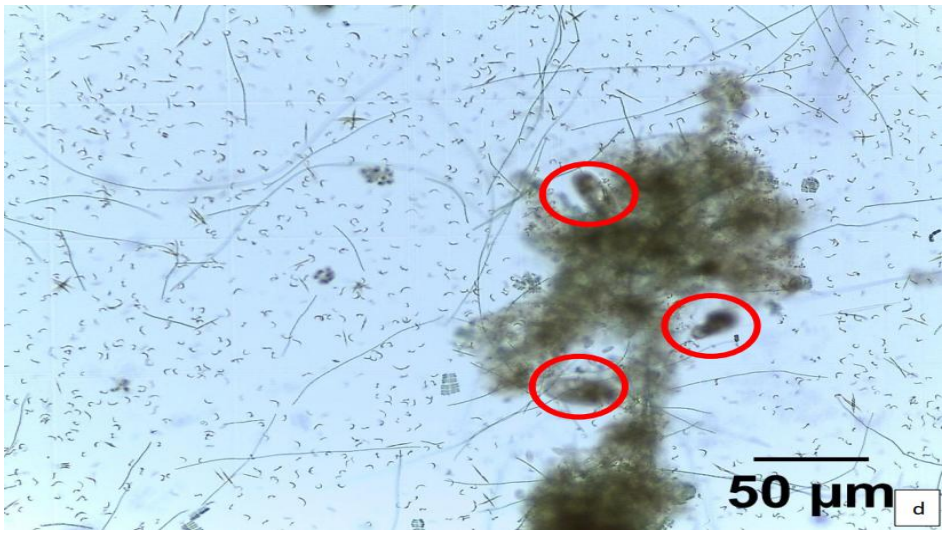


Figure 3 (d). The presence of microzooplanktonic grazers in the freshwater sample viewed under microscope Leica DM750 with 10x magnification.

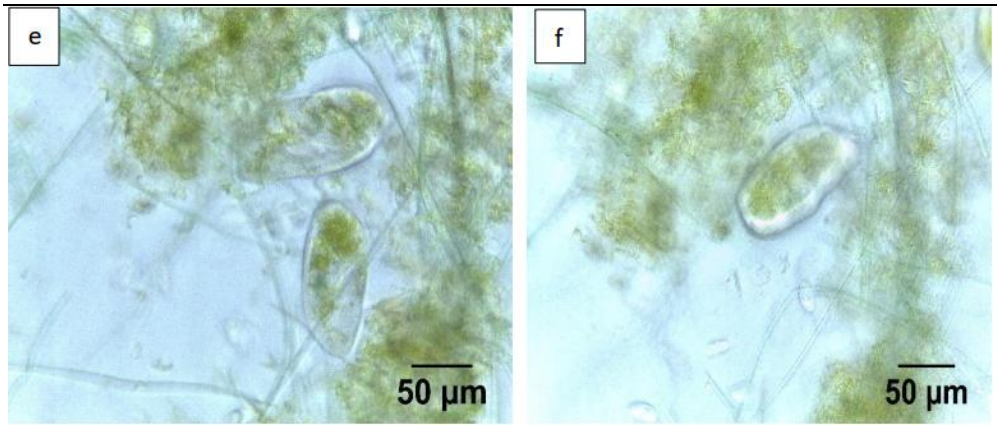


Figure 3 (e) and Figure 3 (f) Close-up image of the presence of microzooplanktonic grazers in the freshwater sample at 100x magnification.

In respond to the presence of microzooplanktonic grazers, it has become more challenging to isolate the potential *Chlorella* sp. from the freshwater sample which was dominant by other microalgae species. According to Day (2017), the possible microzooplanktonic grazers found could be *Poterioochromona*s sp. and *Pseudobodo* sp. which are a kind of flagellate, the reason is because the microzooplanktonic grazers have flagella and would feed on *Chlorella* sp.as these species are natural predator of *Chlorella* sp. The presence of predator in the freshwater sample as well as the competition between other microalgae species would eliminate the potential *Chlorella* sp. Hence, the freshwater sample were then diluted and being sub-cultured on BG-11 media agar plate to enrich and isolate the potential *Chlorella* sp. in the freshwater sample.

The cellular morphological identification of the *Chlorella* Sp. Was conducted after the population of the species have grown dense to enable the identification to be more precise. General morphology of the green algae strains was spherical such as the elliptical *Chlamydomonas* sp., ellipsoidal and cylindrical *Chlorella sorokiniana (*Kim *et al.*, 2014*)*. The microalgae species that were isolated from the Sembrong Barat dam was suspected to be *Chlorella* sp. based on its physical characteristics which is rounded in shape, green in colour and unicellular. According to the research by Kim *et al., (*2014*)*, the freshwater samples collected in Sembrong Barat Dam was screened using the LG Sonic MPC-Buoy device which has the ability to monitor water quality using sonic wave. In addition, the device also has the ability to detect the amount of chlorophyll-α, phycocyanin, water clarity, pH, dissolved oxygen levels and temperature apart from eliminating the microalgae. According to him again, the ultrasonic device was used to reduce the overpopulated microalgae in the Sembrong Barat Dam and the presence of *Chlorella* sp. were detected at that time. Consequently, the possibility of this microalgae to inhabit the location nearby is high as the size of the dam is much for the ultrasonic wave to fully

covered and eliminate the microalgae effectively. Figure 4 (a) shows the isolated *Chlorella* sp. while Figure 4 (b) shows the *Chlorella* sp. identified using the LG Sonic MPC-Buoy device.

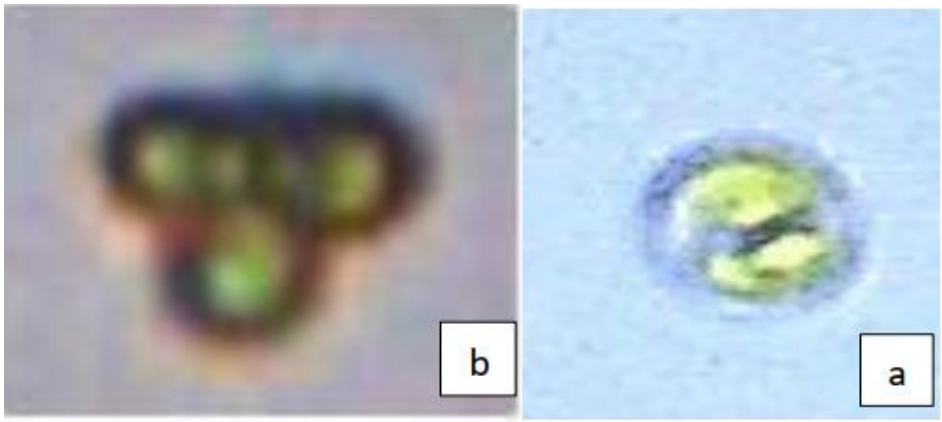


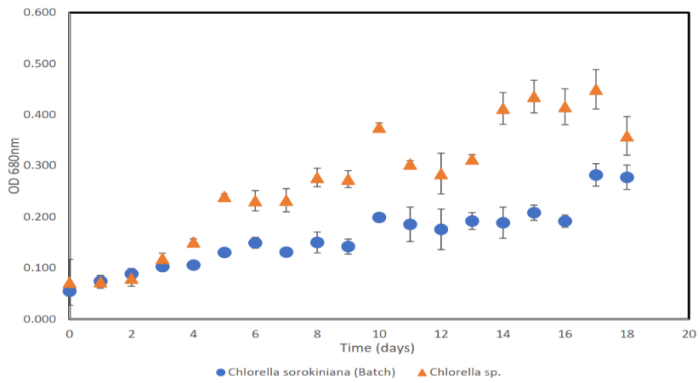
Figure 4 (a) shows the cultured *Chlorella* sp. isolated from the freshwater sample. while (b) show the *Chlorella* sp. detected from the freshwater sample using LG Sonic MPC-Buoy device *(*Kim *et al.*, 2014*)*.

The size of the isolated microalgae species was around 10µm diameter which was shown earlier on the Figure 1. using microscope Leica DM750 with 100x magnification and green in colour. Additionally, in figure 4 (a), there was an inner barrier within the cell. According to Safi (2014), this would happen when *Chlorella* sp. undergoes its asexual reproduction, the mother cell would form an inner barrier between its daughter cell and which would form a new cell wall after the reproduction process is completed. In short, based on the morphology and the supporting evidence of microscopic morphology cellular observations and previous studies, there is much higher possibility for the isolated microalgae to be identified as *Chlorella* sp.

Comparing the growth performance of freshwater isolated *Chlorella* sp. and *Chlorella sorokiniana* in the conical flasks.

Figure 5 shows the comparison of growth profile between freshwater isolated *Chlorella* sp. and *Chlorella sorokiniana* that were cultured in the different conical flasks with triplicate. Both species were supplied with the same growth media which is BG11, and cultured for 18 days under the same growth conditions such as light intensity and inoculum size at 3000 Lux and 10% respectively. In addition, samples of the *Chlorella* sp. and *Chlorella sorokiniana* were collected daily and being measured by spectrophotometer (UV-Vis) at 680nm to obtain respective optical densities. From the growth profile, the specific growth rate (µ), specific maximum growth rate (µmax) were calculated from the logarithmic growth phase over 3-4 days batch culture in each experiment. Next, dry cell weight (g/L) of each cultivation was calculated

by using Microsoft excel version 2016 software, and OriginPro2021 software



were used to plot the following graph.

Figure 5 The comparison of growth profile between freshwater isolated *Chlorella* sp. And *Chlorella sorokiniana.*

The graph showed that the *Chlorella* sp. well growth compared to *Chlorella sorokiana*. Hence *Chlorella* sp. was selected for further investigation to find the other beneficial parts. The growth performance was generated from different parameters such as aeration, pH, light intensities, and different inoculation size.

Conclusion

As conclusion, the microalgae collected were successfully isolated from freshwater sample at Ayer Hitam, Johor and morphologically identified as *Chlorella* sp. after sub-cultured for 18 days. Consequently, the growth profile of the freshwater isolated *Chlorella* sp. and the coal-fired power plant environment isolated *Chlorella sorokiniana* were obtained after cultured for 18 days. As the result, the *Chlorella* sp. was the strain with better performance compared to *Chlorella sorokiniana* were then cultured in the 2 Litre photobioreactor and the growth affecting factors were optimised. Generally, a complete growth profile with lag phase, exponential phase and stationary phase of *Chlorella sorokiniana* was able to obtained after culture for at least 14 days. Nevertheless, there are still lots of space for improvement such as adding lipid extraction and lipid profiling to further investigate the lipid accumulationin the *Chlorella* sp. Likewise, molecular identification such as 18s rRNA identification could be carried out to identify the isolated species which is a more precise and accurate approach.

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