The Growth Performance of Freshwater *Chlorella* sp. and *Scenedesmus* sp. in Different Media

Nur Zuliyana Mohd Shukri, Hasnun Nita Ismail, Tay Chia Chay, Abdul Mutalib Md Jani

**ABSTRACT**

**Background:** Medium is one of the major factors for controlling the growth performance of microalgae. Growth performances of microalgae are very important for production of essential nutrients by these organisms. In this study, two different species of freshwater microalgae, *Chlorella* sp. and *Scenedesmus* sp., were cultured in different media at 28±2 °C under light intensity of 1900 lux with 12D: 12L hour photoperiod for a month. **Objective:** This study was performed to determine the effect of medium on the growth performance of *Chlorella* sp. and *Scenedesmus* sp. in BBM and Chu medium. **Results:** The study indicated that *Chlorella* sp. and *Scenedesmus* sp. showed varied growth pattern in different culture media. However, there were no significant changes (P>0.05) in the growth rates of two species microalgae due to the same genera which is *Chlorophyta*. **Conclusion:** As a final point, these two microalgae species are able to survive in BBM and Chu medium. In addition, these optimized growth medium will be used to increases the biomass yield for other application due to the fast growth.

**INTRODUCTION**

Microalgae are diverse group of prokaryotic and eukaryotic photosynthetic organisms. They are widely found in both marine and freshwater environments and can grow quickly due to their simple structure (Ilavasi et al., 2011). Microalgae are able to grow when all the necessary growth factors are sufficient in the environments. The factors include optimal light intensity, pH stability, consistent temperature, high availability of nutrients (Shay et al., 1987). Even in the adequate growth factors each species of microalgae display different growth performance for example, *Chlorella* sp. requires the existence of sodium carbonate and silicate for their higher growth (Sharma et al., 2013). While *Scenedesmus* sp. is able to be grown in nutritionally rich medium such as in BBM (Geldenhuys et al., 1988).

*Chlorella* sp. is the green single celled microalgae characterized by spherical body shaped. The size of this species is about 5 μm in diameter. *Chlorella* sp. has been cultured for commercial production of health foods or supplement, pharmaceutical and cosmetics industry (Sankar and Ramasubramanian, 2012). In general, *Scenedesmus* sp. nonmotile colonial green microalgae consisting of cells aligned in a flat plate. The size of this species is about 8.5 μm in width and 13.5 μm in length. *Scenedesmus* sp. has been cultured for live feed aquatic organisms (Mayeli et al., 2004). These microalgae species are enriched in nutritional value. The nutritional value of *Chlorella* sp. and *Scenedesmus* sp. are due to their high content of protein, polysaturated fatty acids (PUFA), carotenoids, bioactive compounds, and vitamins (Chu, 2012). Each species of microalgae produces different ratio of lipids, carbohydrates, carbohydrates and proteins for varies application (Ilavasi et al., 2011).

The suitable culture medium is the major factor to enhance the growth of microalgae (Gong and Chen, 1997). The most favorable of culture medium is strongly depends on various chemical composition in the medium and several medium are available for cultivation of microalgae (Borowitzka, 2005). Culture media was integrated with trace metals, vitamins, organic and inorganic salts. These elements such as Nitrogen (N), Phosphorus (K), Phosphate (P), Magnesium (Mg), Calcium (Ca), Sulfur (S), Iron (Fe), Manganese (Mn), and Zinc (Zn) which helps the growth of microalgae. In general, Bold’s Basal Medium (BBM) and Chu medium were found to greatly influence the growth of freshwater microalgae (Ilavasi et al., 2011). These findings reveal that some species of microalgae may require certain factors of specific factors to enhance the growth performance.

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The aim of this present investigation, the growth performance of two different species microalgae which was optimally cultured under laboratory condition, *Chlorella* sp. opposed the species that was isolated from wild environment which is *Scenedesmus* sp. In assessing the growth performance of these two microalgae, we used two commercial freshwater media which are Bold’s Basal Medium (BBM) and Chu medium.

1. Methodology:

Samples:

*Scenedesmus* sp. was collected using plankton net with 153 μm mesh size and was isolated from freshwater body at Tasik Ilham, Perlis Malaysia. The sample was optimized with both media under laboratory condition until 3rd generations. *Chlorella* sp. was earned from laboratory population provided by Universiti Tun Abdul Razak (UTAR) Microalgae Sdn Bhd (UMSB).

Culture and growth condition of microalgae:

A modified version of Bold’s Basal Medium (BBM) (Bischoff and Bold, 1963) and Chu Medium (Chu, 1942; Stein, 1973) were employed in the experiment. These media contains macro and micro nutrients necessary for the growth of microalgae. The stock solutions were prepared and stored in the refrigerator at approximately ±3ºC until the final media preparation. All the media compositions were sterilized using autoclave (TOMY) at 121ºC for 15 minutes at 15 psi.

Then the growth medium was prepared based on their compositions in 2000 mL glass container. The aeration was supplied and the air was filtered using nylon filter with 0.45 μm pores size. Microalgae were inoculated into each glass container from stock with the equal concentration density (±0.800 ABS) at 690 nm wavelength using spectrophotometer (SPECTRONIC 200, Version 2.04) (Rundquist et. al., 1996). They were cultured at 28±2 ºC under light intensity of 1900 lux with 12D: 12L hour photoperiod for a month.

Determination of growth curve and growth rate:

The growth media were assessed at exponential phase on daily basis to obtain the growth curve. Everyday, 10 μL of microalgae samples were quantified using haemocytometer under light microscope (OLYMPUS, CX22LED) within a month. The cells of microalgae were estimated according to the following formula (Clesceri et al., 1989; Toyub et al., 2008):-

$$\text{Number of cells per milliliter} = \frac{\text{No. of cells in counted area}}{\text{Volume of counted area (0.001 x 4)}} \times \text{Dilution factor}$$

Then, the enumerations of microalgae were converted to biovolume. The unit of biovolume was used in this study as a standard measurement to offset the error due to the different size of microalgae. Linear dimensions were measured according to taxonomic information and the shape of microalgae. The length and width of the cells were quantified according to the formula Sun and Liu, 2003. The biovolume in mm$^3$/L of each species in the sample was calculated using the following equation:-

$$\text{Biovolume} = \frac{n \times \text{Vol}}{1 \times 10^6}$$

Where $n$ is number of cells in a sample of microalgae (cells/mL), Vol is the volume of each cells (μm$^3$) and $1 \times 10^6$ is a unit conversion from (μm$^3$/mL) to (mm$^3$/L).

The growth rate of microalgae was expressed in terms of growth rates using the following equation:-

$$\text{Growth Rate (μ day$^{-1}$)} = \frac{\ln (N_2 / N_1)}{t_2 - t_1}$$

Where, $N_1$ is biomass at time harvest ($t_1$) and $N_2$ is biomass at time of ($t_2$) respectively (Guillard, 1973). $N_1$ was taken at the starting point of exponential phase and $N_2$ was taken at the starting point of stationary phase in the growth phase of microalgae.

Determination the chlorophyll:

The chlorophyll from microalgae was extracted using acetone according to Sartory & Grobberlaar (1984); Simon & Helliwell (1998); Schumann et al., (2005). Firstly, 50 mL of microalgae sample was centrifuged at 3500 rpm for 5 minutes. The supernatant was discarded and the residual microalgae was collected. Then, 10 mL of acetone was added and the extraction was sonicated for 10 minutes to disrupt the microalgae cells. Afterwards, 3 mL of extracted microalgae were put in the glass cuvette and assessed using spectrophotometer at 3 different wavelength according to the formula Jeffrey and Humphrey (1975).
The concentrations of chlorophyll were calculated using the following generalized equation:

\[ C_a = \frac{C_e (a, b \text{ or } c) \times \text{extract volume (L)} \times \text{DF}}{\text{Sample volume (L)} \times \text{cell length (cm)}} \]

Where:
- \( C_e \) = concentration (mg/L) of chlorophyll
- \( C_{e(a, b \text{ or } c)} \) = concentration (mg/L) of chlorophyll
- \( \text{Extract volume} \) = volume of solvent extraction
- \( \text{DF} \) = dilution factors (if any)
- \( \text{Sample volume} \) = sample used
- \( \text{Cell length} \) = optical path of cuvette used (1 cm)

**Statistical analysis:**

The growth rates of microalgae were quantified from the end of lag phase to the beginning of stationary phase. The statistical analysis was done using the SPSS software (Version 21). The difference of growth rates among microalgae in different media were tested using T-TEST and two-way ANOVA in the evaluation of differences in the mean values. The significant level is set up at \( P<0.05 \).

**3. Results:**

*The growth curve of Chlorella sp. and Scenedesmus sp. in different medium:*

Fig. 1: Growth curve of *Chlorella* sp. based on Biovolume (mm3/L) in different media (a) Chu Medium (b) BBM.

Fig. 2: Growth curve of *Scenedesmus* sp. based on Biovolume (mm3/L) in different media (a) Chu Medium (b) BBM.

Fig. 1 showed the growth curve of *Chlorella* sp. in Chu Medium (a) with \( r^2 = 0.9034 \) and BBM (b) with \( r^2 = 0.9915 \). In Chu medium, the duration of lag phase was approximately 3 days. Exponential phase was started at day 3\(^{\text{rd}}\) until day 13\(^{\text{th}}\) and takes about 10 days. Stationary phase was started from day 14\(^{\text{th}}\) until day 25\(^{\text{th}}\) and takes about 12 days. The maximum cells count and biovolume of *Chlorella* sp. cells in BBM is \( 1.66 \times 10^7 \).
cells/mL and 1085.5 mm³/L on day 21st. In the meantime in BBM, the duration of lag phase was approximately 4 days. Exponential phase was started at day 4th until day 15th and takes about 11 days. Stationary phase was started from day 15th and the cells showed no decrement until day 30th. The maximum cells count and biovolume of *Chlorella* sp. cells in Chu medium is $1.39 \times 10^7$ cells/mL and 907.7 mm³/L on day 23rd.

<table>
<thead>
<tr>
<th>Variables</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Sig.</th>
</tr>
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<tr>
<td>Medium * Microalgae</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lag phase</td>
<td>1</td>
<td>0.063</td>
<td>0.153</td>
<td>0.786</td>
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<tr>
<td>Exponential phase</td>
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<td>3.748</td>
<td>1.463</td>
<td>0.258</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag phase</td>
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<td>0.274</td>
</tr>
<tr>
<td>Exponential phase</td>
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<td>5.200</td>
<td>0.052</td>
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<tr>
<td>Microalgae</td>
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<td></td>
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<tr>
<td>Lag phase</td>
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<td>0.946</td>
<td>21.834</td>
<td>0.002*</td>
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<tr>
<td>Exponential phase</td>
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<td>2.058</td>
<td>0.815</td>
<td>0.393</td>
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</table>

*significant different at P<0.05

In the meantime, Fig. 2 showed the growth curve of *Scenedesmus* sp. in Chu Medium (a) with $r^2 = 0.7871$ and BBM (b) with $r^2 = 0.9936$. In Chu medium, the duration lag phase was about 3 days. Exponential phase was started at day 3rd until day 11th and takes about 8 days. Stationary phase was started from day 11th until day 24th and takes about 13 days. The maximum cells count and biovolume of *Scenedesmus* sp. cells in Chu medium is $4.47 \times 10^5$ cells/mL and 228.1 mm³/L on day 19th. In the meantime in BBM, the duration of lag phase was about 4 days. Exponential phase was started at day 3rd until day 11th and takes about 8 days. Stationary phase was started from day 11th and the cells showed no decrement until day 30th. The maximum cells count and biovolume of *Scenedesmus* sp. cells in BBM is $5.34 \times 10^5$ cells/mL and 272.8 mm³/L on day 25th and 29th respectively.

Based on the statistical analysis, there was no interactive effect between the type of microalgae and medium on the duration time taken for lag and exponential phases (Two-way ANOVA, $P>0.05$; Table 1). The duration time taken for lag phase was significantly different between microalgae (Two-way ANOVA, $P<0.05$; Table 1). However, the duration time taken for lag phase was insignificant different between media (Two-way ANOVA, $P>0.05$; Table 1). Otherwise, the duration time taken for lag and exponential phases was unaffected between both microalgae (Two-way ANOVA, $P>0.05$; Table 1) and media (Two-way ANOVA, $P>0.05$; Table 1).

**Growth rates of *Chlorella* sp. and *Scenedesmus* sp. in different media:**

Based on the result, the growth rate of *Scenedesmus* in Chu medium (0.25±0.02 μ/day) was higher than *Scenedesmus* sp. culture in BBM (0.22±0.04 μ/day) (Fig. 3). While, the growth rate of *Chlorella* sp. in BBM (0.27±0.02 μ/day) was higher than in Chu medium (0.26±0.01 μ/day). However, statistical analysis showed that

**Fig. 3:** The Growth rates of *Scenedesmus* sp. and *Chlorella* sp. in different medium.

<table>
<thead>
<tr>
<th>Variables</th>
<th>t</th>
<th>df</th>
<th>MD</th>
<th>Sig.</th>
</tr>
</thead>
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<td>Microalgae * BBM</td>
<td>0.904</td>
<td>4</td>
<td>0.047</td>
<td>0.397</td>
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<tr>
<td>Microalgae * CHU</td>
<td>0.134</td>
<td>4</td>
<td>0.003</td>
<td>0.900</td>
</tr>
<tr>
<td><em>Scenedesmus</em> sp. * media</td>
<td>-0.725</td>
<td>4</td>
<td>-0.037</td>
<td>0.598</td>
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<td><em>Chlorella</em> sp. * media</td>
<td>0.401</td>
<td>4</td>
<td>0.100</td>
<td>0.799</td>
</tr>
</tbody>
</table>
media was insignificantly different the growth rate of Chlorella sp. and Scenedesmus sp. (T-TEST, \(P>0.05\); Table 2).

*The total chlorophyll of Chlorella sp. and Scenedesmus sp. in different media:

![Bar chart](image)

**Fig. 4: Total Chlorophyll of Scenedesmus sp. and Chlorella sp. in different medium.**

<table>
<thead>
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<th>Table 3: Results of T-Tests for the total chlorophyll. df – degree of freedom, MS – Mean square.</th>
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<td>Chlorophyll(^*) medium</td>
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<tr>
<td>Chlorophyll(^*) microalgae</td>
</tr>
<tr>
<td>Scenedesmus (^*) Chlorella</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4: Results of T-Tests for the maximum cells. df – degree of freedom, MS – Mean square.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
</tr>
<tr>
<td>Maximum cells(^*) medium</td>
</tr>
<tr>
<td>Maximum cells (^*) microalgae</td>
</tr>
<tr>
<td>Scenedesmus (^*) Chlorella</td>
</tr>
</tbody>
</table>

\(^*\)significant different at \(P<0.05\)

Fig. 4 showed the total chlorophyll of Chlorella sp. was higher in BBM (4.05±0.30 mg/L) than in Chu medium (3.22±0.37 mg/L). It also correspondingly with Scenedesmus sp. that was higher in BBM (1.03±0.07 mg/L) than in Chu medium (0.75±0.07 mg/L). Based on the statistical analysis, the total chlorophyll were significantly different between these two microalgae (T-Test, \(P<0.05\)). Meanwhile, there was no significant different between both microalgae and the two different media (T-Test, \(P>0.05\)). The maximum cells of microalgae were quantified at stationary phase (Table 4). The maximum cells of Chlorella sp. was greater in Chu medium (1.59 x 10\(^{7}\) ± 2.38 x 10\(^{5}\) cells/mL) rather than in BBM (1.32 x 10\(^{7}\) ± 1.98 x 10\(^{5}\) cells/mL) whereas Scenedesmus sp. was greater in BBM (5.19 x 10\(^{5}\) ± 4.75 x 10\(^{3}\) cells/mL) rather than in Chu medium (4.27 x 10\(^{5}\) ± 6.10 x 10\(^{3}\) cells/mL). The maximum cells was significantly different between these two microalgae (T-Test, \(P<0.05\)) but not significantly different between the microalgae with two media.

4. Discussion:

The different of growth performance of Chlorella sp. and Scenedesmus sp. were determined using two inorganic medium which are BBM and Chu medium with varying chemical compositions. Since the sizes of microalgae in this study are dissimilar, these two different species microalgae were enumerated using biovolume. Biovolume and surface area calculations for phytoplankton cells are important for many related ecological parameters (Hillebrand et al., 1999). The two species of microalgae showed the similarities in their growth pattern. Generally, the growth pattern of microalgae presented the “S” shape which known as sigmoid. The growth of microalgae was divided into four phases which are lag, exponential, stationary and senescence phases (Barsanti and Gualtier, 2006).

The lag phase is the period where the cells undergo the adaptation mode after the first inoculation from the stock to new culture condition (Collos, 1986). The results revealed that Scenedesmus sp. took less duration than Chlorella sp. to adapt in the two different media. The duration time are varies at the lag phase due to the several factors including the physiological history of the microalgae cells, inoculation volume, the accurate physiochemical environment; nature and the man-made new culture medium (Swinnen et al., 2004). For the reason that, Chlorella sp. acquires a lot of small single cells and might takes more time to familiarize in new culture environment compared to Scenedesmus sp. After the cells well adapt in the new culture environment the
cells were utilized all the elements in culture media and increased tremendously due to the high rate reproduction at exponential phase. However, the duration of exponential phase is similar in both microalgae.

Later than, the cells were actively divided and start to synthesis their products in stationary phase. In this stage, the cells densities were balanced and no increments occur at this phase due to the limitation of nutrients (Navarro et al., 2010). In addition, both microalgae reach the stationary phase continuous than a month in BBM. After a month, both microalgae undergo senescence phase in Chu medium. Senescence phase occurred due to the severe nutrient depletion. At this stage, the cells were rapidly decreased (Finkel, 2006). This issue occurred owing to the chemicals concentration in BBM that was higher than Chu medium. Under those circumstances, the growth performance of microalgae is mostly relied with the quality of medium used for their cultivation (Lam and Lee, 2012; Prathima Devi et al., 2012). For this reason, both microalgae practically survive longer in BBM. In state of high range nutrients in culture media, microalgae are able to maintain for a long time due to high tolerance with nutrients (Fathurrahman et al., 2013).

The present research discovered the cells density of *Chlorella* sp. is higher than the growth of *Scenedesmus* sp. This reason is due to the total biovolume of these different species of microalgae. *Chlorella* sp. has an average size of 5 μm in diameter while *Scenedesmus* sp. is about 13.5 μm in length and 8.5 μm in width. Total surface area per volume of *Chlorella* sp. is higher than *Scenedesmus* sp. Thus, *Chlorella* sp. can takes nutrients rapidly rather than *Scenedesmus* sp. The growth of *Chlorella* sp. cultured in Chu medium reached the highest number of cells compared in BBM (Ilavasi et al., 2011; Sharma et al., 2011). Meanwhile, *Scenedesmus* sp. reached the highest number of cells when cultured in BBM compared in Chu medium (Ilavarasi et al., 2011; Al-Shatri et al., 2014). It is because the larger cells required more nutrients for growth and reproduction compared to the smaller size of microalgae (Fathurrahman et al., 2013). Nevertheless, these two microalgae does not show any decrement until a month culturing and more survive in BBM. Overall, the statistic results explained that the growth performance of *Chlorella* sp. and *Scenedesmus* sp. in two different media are not significantly changes. These present results not illustrate significantly changes possibly due to the same group of microalgae. The reaction of microalgae genera on the consumption of the cultivation medium were based on its specific biological requirement and thus it differed between genera (Collect et al., 2011; Fathurrahman et al., 2013).

Assessment of total chlorophyll was conducted using microalgae at stationary phase. The maximum cells also were quantified at stationary stage. At this stage, microalgae start to produce bioactive compound such as Polysaturated Fatty Acids (PUFAs) (Mata et al., 2010). The present result revealed that total chlorophyll in *Chlorella* sp. was significantly higher than total chlorophyll in *Scenedesmus* sp. *Chlorella* sp. have the greater maximum cells and their size was smaller compared to *Scenedesmus* sp. (Ilavarasi et al., 2011). On the subject of maximum cells, it was leaded to increase the total chlorophyll content in microalgae owing to the number of chloroplast content in each cell of microalgae. Regarding to this statement, higher content of chloroplast will upsurge the photosynthesis process and produced supplementary bioactive compounds.

5. Conclusion:

The present work investigated the effect of BBM and Chu medium to the growth performance and total chlorophyll content of *Chlorella* sp. and *Scenedesmus* sp. Ultimately, in this present experiment showed that BBM and Chu medium were practically suitable to culture the microalgae in group chlorophyta. As a recommendation, this study will be pursued with the PUFAs analysis. PUFAs possibly relate with the total chlorophyll content due the photosynthesis process occurred in chloroplast contain in each cells of microalgae.

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