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Growth and Lipid Production of *Isochrysis galbana* in an Upscale Cultivation System

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ABSTRACT Microalgae for biofuel production require further research and development to be economically viable, especially in terms of cost and biomass production. These include the need to optimize the favorable growth conditions for low-cost but large-scale cultivation. This study aims to determine the best initial biomass concentration of *Isochrysis galbana* on a pilot scale cultivation system. *Isochrysis galbana* was cultured for 69 days in an upscaled 2-liter Erlemenmeyer flask with a variety of initial biomass concentrations using the previously established stock culture (250 ml) at 25°C room temperature, 16:8 light/dark cycle, and 135 μmol/m^2/s light intensity. The initial biomass concentration was optimized from a range of 10^3 cells/ml to 10^4 cells/ml, 10^5 cells/ml, and 10^6 cells/ml. The cell density was calculated every three days to determine the growth curve, and the lipid content was measured weekly throughout the cultivation cycle. The results show that the 10^6 cells/ml initial concentrations produced the highest growth, but the 10^4 cells/ml initial concentration produced the highest lipid content. This finding indicates that a higher initial concentration might be better for cell growth in upscale cultivation, but not for lipid production, which may be due to the presence of threshold nutrients.

KEYWORDS: *Isochrysis galbana*, initial biomass concentration, cell growth, lipid production, upscale cultivation
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INTRODUCTION

The increasing demand for renewable energy and severe environmental pollution issues make the search for a long-term solution critical. The importance of identifying potential renewable sources for sustainable energy production has recently gained momentum in the global market, with the goal of addressing these issues, especially those related to energy security and climate change (Ramkumar & Kirubakaran, 2016). Microalgae has the potential to be used in the manufacture of biofuels. Microalgae biofuel production is also cost-competitive to fossil fuels, requires no additional land, improves air quality by removing atmospheric carbon dioxides, and uses minimal water (Zhu *et al.*, 2016). Microalgae biofuels, on the other hand, have some disadvantages, such as low biomass yield, low lipid content in the cells, and small cell size, all of which make the harvesting process very costly. These limitations can be overcome by developing more advanced cultivation, harvesting, and drying techniques, as well as genetic engineering of metabolic pathways for improved growth rate and lipid content (Shokravi *et al.*, 2020).

In order for algal biofuel to become a more viable energy source, species selection is one of the most critical factors in increasing yield and productivity. Species selection is important because it marks the beginning of any algal mass cultivation, based on their characteristics such as fast-growing, productivity and suitability to the local climatic conditions (Krishnan *et al.*, 2015). *Isochrysis galbana* is a marine microalga with a high potential for improved biofuel production (Cordoba-Matson *et al.*, 2013). *Isochrysis galbana* is a flagellated microalga belonging to the phylum Haptophyta. This genus has been used as animal feed in aquaculture for centuries due to its comparatively fast growth rate, small size, non-toxic nature, and superior nutritional value (Cordoba-Matson *et al.*, 2013). Temperature, pH, salinity, nitrogen availability, and light are all factors that influence microalgae cell growth and biochemical composition (Costard *et al.*, 2012). For indoor culturing, *I. galbana* grow efficiently in Walne's media with temperature range 23-25°C, 5 g/L

nitrogen concentration, 135 μmol/m^2/s light intensity and reach mid-log phase within 14 days of cultivation (Andrew, 2014). According to Huerlimann *et al.* (2010), variations in irradiance are common in various cultivation systems, whether indoor or outdoor, and that they have a significant physiological and biotechnological impact on the biochemical composition of microalgae.

When it comes to microalgae production for industrial applications, upscaling is a particularly challenging process. One of the most difficult tasks is to prepare a suitable initial microalgae biomass with adequate concentration, as well as to plan and design an efficient system for propagating the microalgae, as the quality of the initial biomass has a significant impact on both the growth and productivity of the cultures (Bohutskyi *et al.*, 2016). According to Borowitzka & Vonshak (2017), scaling up algal cultures to the very large volumes required for commercial production is a complex task that necessitates the use of highly skilled and experienced personnel. Optimizing the process of producing suitable biomass concentration and quality for large-scale culture, whether in outdoor ponds or indoor photobioreactors, should be prioritized to minimize the time and cost of achieving maximum productivity. It is one of the most crucial factors in algal culture because it has a major impact on microalgal growth, lipid accumulation, and metabolism efficiency (Lu *et al.*, 2013). This study aims to examine the effects of initial biomass concentration on the growth and lipid production of *I. galbana* in an upscaling cultivation system.

METHODOLOGY

Microalgae and Cultivation Condition

The stock culture of *I. galbana* microalgae was obtained from the Borneo Marine Research Institute, University Malaysia Sabah. The culture was maintained in a 250 ml stock in Walne's media at 25°C room temperature, 16:8 light/dark cycle, and 135 μmol/m^2/s light intensity.

Upscaling Cultivation Conditions

Isochrysis galbana was cultured with a variety of initial biomass concentrations in a pilot scale 2-liter Erlenmeyer flask from the previously established stock culture (250 ml). The biomass was obtained based on the maximum cell density growth of the stock culture by cell counting Haemocytometer (v/v). Three replicates of 1-liter culture volumes of *I. galbana* were inoculated with cell densities of approximately 10^3 cells/ml, 10^4 cells/ml, 10^5 cells/ml, and 10^6 cells/ml initial concentrations from the stock culture at the mid-exponential phase (day 14). The cell density was measured using a direct microscopic count with a 0.1-mm-deep Neubauer haemocytometer v/v and a light microscope. All cultures were held at 25°C room temperature for 69 days, with a 16:8 light/dark cycle and a light intensity of 135 μmol/m^2/s, and the growth and lipid production of the cultures were analyzed.

Growth Analysis

The cultures were sampled every three days, and the density of cells was determined using the Equation (1) while the specific growth rate was calculated using Equation (2):

$$\text{Density of cells (cell/ml)} = \text{Average count per square} \times \text{dilution factor} \times 10^4 \quad (1)$$

$$\mu = \ln(N_2\text{-}N_1)/t_2\text{-}t_1 \quad (2)$$

where N_2 and N_1 are the number of cells (N) at the start (t_2) and end (t_1), respectively, of the logarithmic growth phase (Wood *et al.*, 2005).

Lipid Extraction and Analysis

Microalgal lipid content was measured once a week for up to 69 days. The dry algal biomass was subjected to extraction using a 1:1:1 mixture of chloroform, methanol, and distilled water (Breil *et al.*, 2017). The mixtures were vortexed before being left on the bench for 1 to 2 h. Following that, the samples were centrifuged for 5 min at 5500 rpm to form layers. The top layer (methanol and water) was discarded, while the bottom layer (chloroform and lipid) was transferred to a pre-weighed microcentrifuge tube and dried for 1.5 h using speed vacuum (V-AQ) to remove excess methanol. The final lipid weight was calculated according to Xu & Boeing (2014) using Equation (3) and lipid productivity was calculated using Equation (4).

$$\text{Lipid content (\%)} = [\text{lipid extract weight (g)} / \text{dry biomass (g)}] \times 100 \quad (3)$$

$$\text{Lipid productivity (mg/L/day)} = \text{Lipid content value} \times \text{biomass productivity} \quad (4)$$

RESULTS AND DISCUSSION

Figure 1 shows pictures of *I. galbana* observed with a light microscope and used in the present study. The growth pattern of *I. galbana* is as depicted in Figure 2. Based on the figure, the growth patterns for all initial biomass concentrations were nearly identical, with the exception of the smallest concentration, which remained in the log-phase until day 60, but the other concentrations entered death phase between 45 and 57 days of cultivation.

Table 1 shows that the growth rate in 10^6 cells/ml was faster than the growth rate of other initial biomass concentrations such as 10^3 cells/ml, 10^4 cells/ml, 10^5 cells/ml. The most productive initial biomass concentration was 10^6 cells/ml, which had a specific growth rate of 0.42 day^{-1}, while the least productive concentration was 10^3 cells/ml, suggesting that 10^6 cells/ml was the best initial biomass concentration for optimal growth in a 1-liter culture volume. The mid-log phase began around day 15 for most initial biomass concentrations, and cells continued to expand until the stationary phase began around day 36. With the exception of culture with the smallest initial biomass concentration, which continued to grow, most initial biomass concentrations began to decline around day 54.

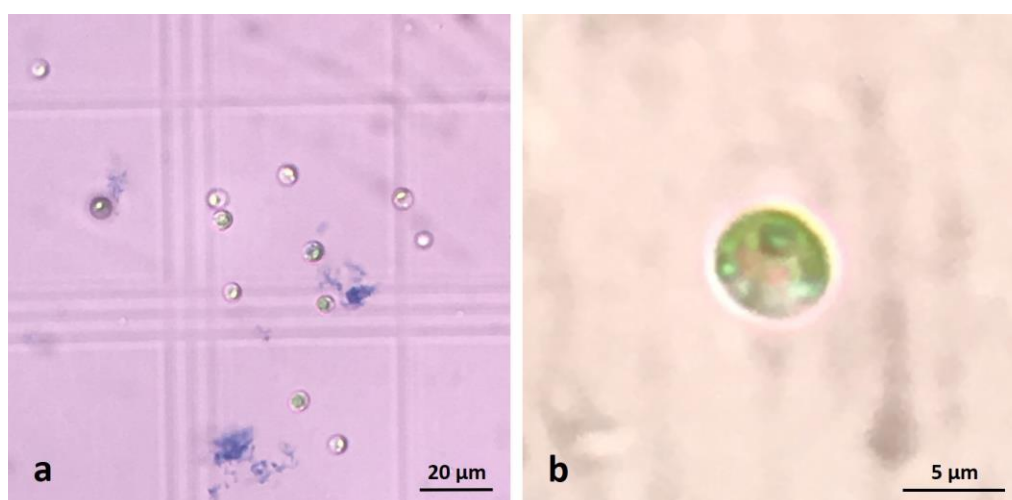

Figure 1. Samples of *Isochrysis galbana* observed at 40× (a) and 100× (b) magnifications with a light microscope.

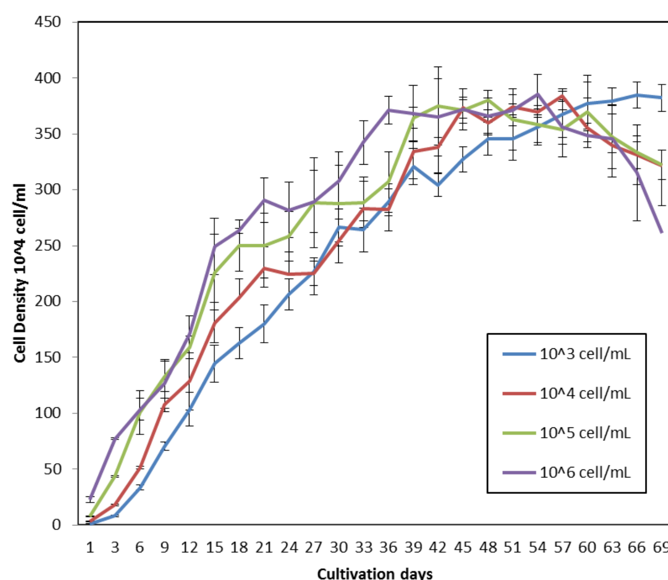

Figure 2. Growth profile of *Isochrysis galbana* in different initial biomass concentrations. Error bars represent the standard deviation (n=3).

Table 1. Specific growth rate of *Isochrysis galbana* in different initial biomass concentrations.

Initial biomass concentration	Specific growth rate, μ (day^{-1})
10^3 cells/ml	0.23 ± 0.03
10^4 cells/ml	0.35 ± 0.02
10^5 cells/ml	0.36 ± 0.01
10^6 cells/ml	0.42 ± 0.01

In general, the initial biomass correlates with the number of cells that will replicate, resulting in biomass production (Gani *et al.*, 2006). In contrast to high initial biomass concentrations, low concentrations are more susceptible to ammonia inhibition (Li *et al.*, 2017). An excessive initial biomass, on the other hand, can cause growth-limiting stresses due to nutrient and light limitations. As a result, selecting the proper initial biomass concentration can help in the production of high biomass and lipid content (Li *et al.*, 2017). However, a few studies have shown that a high growth rate in microalgae did not always promote a higher lipid content due to their culture condition and nutrient composition (Zarrinmehr *et al.*, 2020).

While a higher initial biomass concentration resulted in better growth than a lower concentration, 10^4 cells/ml provided more lipid content than other higher biomass concentrations, as shown in Figure 3. The results showed that lipid production in *I. galbana* follows a similar pattern across all initial biomass concentrations, with the highest lipid production occurring in week 6. The culture is in the end of the exponential phase and the beginning of the stationary phase at week 6, resulting in a high lipid content (Table 2). According to the findings, a 10^4 cells/ml initial concentration produced the highest lipid content and productivitiy of 67.7% and 163.7 mg/L/day respectively. The initial concentration of 10^6 cells/ml had the lowest lipid content and productivity, with 53.37% and 117.4 mg/L/day respectively.

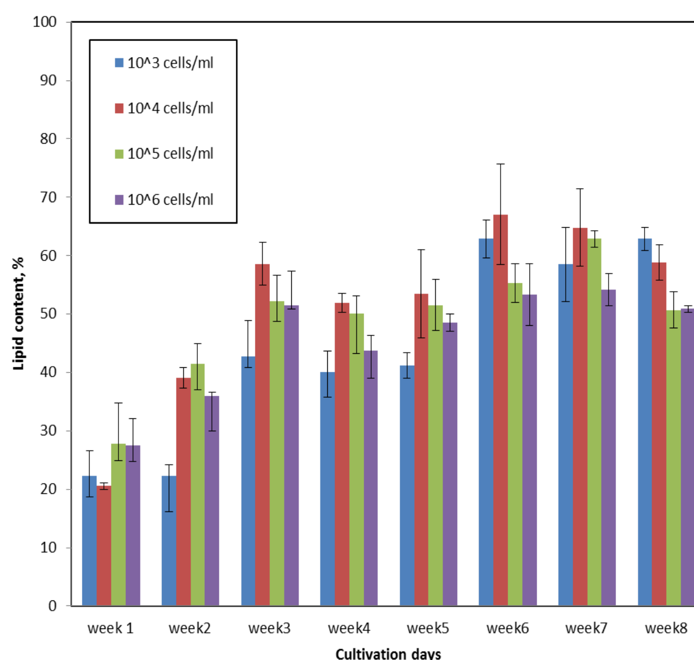

Figure 3. Lipid content (DW%) of *Isochrysis galbana* in different initial biomass concentrations for eight weeks of cultivation. Error bars represent the standard deviation (n=3).

Table 2. Lipid content (DW%) and productivity (mg/L/day) of *Isochrysis galbana* in different initial biomass concentrations at week 6.

Initial biomass concentration	Lipid content (DW%)	Lipid productivity (mg/L/day)
10^3 cells/ml	62.87 ± 3.22	132.70 ± 4.82
10^4 cells/ml	67.07 ± 8.55	163.70 ± 11.03
10^5 cells/ml	55.30 ± 3.31	126.10 ± 9.35
10^6 cells/ml	53.37 ± 5.29	117.40 ± 12.92

Lipid content is typically highest in the late exponential phase, and it can either increase or remain constant in the stationary phase. Aside from biomass lipid content and composition, other important fuel properties include kinematic viscosity (KV), cetane number (CN), and oxidative stability (OS). Unsaturated esters have a CN lower than the standard values for biodiesel and petrodiesel recommended by the American Society for Testing and Materials (ASTM) and the European Committee for Standardization (CEN) (Bohutskyi *et al*., 2016). Although a lower initial biomass concentration may have a lower lipid content and average CN, increasing the biomass concentration above 10^5 cells/ml did not improve lipid content or composition in this study, with the initial concentration of 10^6 cells/ml producing the lowest lipid content compared to the 10^5 cells/ml, 10^4 cells/ml, and 10^3 cells/ml. Furthermore, the high biomass concentration enabled microalgae to outcompete other biological contaminants for nutrient and organic carbon in the media, but it did not cause microalgae metabolism to shift towards lipid accumulation (Caprio, 2020). In addition, nitrogen stress is one of the factors that increases lipid content and productivity in microalgal cultures. However, algal cell responses to nitrogen depletion/repletion are determined by the microalgal strain (Sreedharan *et al*., 2018). Feng *et al*. (2011) found that the lipid content of marine microalgae species increased as nitrate concentrations increased. As shown in this study, a higher initial biomass concentration resulted in lower lipid content due to nitrogen depletion, while a lower initial biomass concentration resulted in higher lipid content due to nitrogen repletion. Lu *et al*. (2013) also suggested that dense microalgae cultures produced by high biomass concentrations

would accumulate less energy-storing products, such as lipids, resulting in a lower lipid content. As a result, an optimal initial biomass is required to achieve maximum lipid production in upscaled microalgae cultivation.

CONCLUSION

Growing microalgae necessitates improved culture conditions, especially when scaling up the cultivation system. The possible use of *I. galbana* species as a biofuel source is a promising source of future energy. However, the effectiveness of culturing *I. galbana* in a large scale depends heavily on the initial biomass concentration to achieve better lipid production. The high initial biomass, despite its faster growth rate, does not necessarily imply a high lipid content. This study included information on the growth and lipid content of *I. galbana* in a pilot scale cultivation system. Based on the current data, an initial biomass of 10^4 cells/ml is the most suitable concentration for *I. galbana* culture for biofuel production in a 1-liter culture volume, and week 6 is the best duration for biomass and lipid production as it exhibited the highest lipid content being generated, and data also showed that the cells entered stationary phase on day 36 (approximately week 5) before dying on day 54 (approximately week 7).

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REFERENCES

[1] Andrew, A. R. 2014. *Characterization of Local Microalgae from Sabah for Biofuels Production*. Msc Thesis, University Malaysia Sabah, Malaysia.

[2] Bohutskyi, P., Kligerman, D. C., Byers, N., Nasr, L. K., Cua, C., Chow, S., Su, C., Tang, Y., Betenbaugh, M. J. & Bouwer, E. J. 2016. Effects of inoculum size, light intensity, and dose of anaerobic digestion centrate on growth and productivity of *Chlorella* and *Scenedesmus* microalgae and their poly-culture in primary and secondary wastewater. *Algal Research*, 19, 278-290.

[3] Borowitzka, M. A. & Vonshak, A. 2017. Scaling up microalgal cultures to commercial scale. *European Journal of Phycology*, 52(4), 407-418.

[4] Breil, C., Vian, M. A., Zemb, T., Kunz, W. & Chemat, F. 2017. "Bligh & Dyer" and Folch methods for solid-liquid-liquid extraction of lipids from microorganisms. Comprehension of Solvatation mechanisms and towards substitution with alternative solvents. *International Journal of Molecular Sciences*, 18, 708.

[5] Caprio, F. D. 2020. Methods to quantify biological contaminants in microalgae cultures. *Algal Research*, 49, 101943.

[6] Cordoba-Matson, M. V., Arredondo-Vega, B. O. & Carreón-Palau, L. 2013. Evaluation of growth, cell size and biomass of *Isochrysis* aff. *galbana* (T-ISO) with two LED regimes. *The All Results Journals*, 4(1), 7-15.

[7] Costard, G. S., Machado, R. R., Barbarino, E., Martino, R. C. & Lourenço, S. O. 2012. Chemical composition of five marine microalgae that occur on the Brazilian coast. *International Journal of Fisheries and Aquaculture*, 4(9), 191-201.

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[8] Feng, D., Chen, Z., Xue, S. & Zhang, W. 2011. Increased lipid production of the marine oleaginous microalgae *Isochrysis zhangjiangensis* (Chrysophyta) by nitrogen supplement. *Bioresource Technology*, 102(12), 6710-6716.

[9] Gani, P., Sunar, N. M., Matias-Peralta, H., Abdul Latiff, A. A. & Abdul Razak, A. R. 2006. Influence of initial cell concentrations on the growth rate and biomass productivity of microalgae in domestic wastewater. *Applied Ecology and Environmental Research*, 14(2), 399-409.

[10] Huerlimann, R., de Nys, R. & Heimann, K. 2010. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. *Biotechnology and Bioengineering*, 107(2), 245-257.

[11] Krishnan, V., Uemura, Y., Thanh, N. T., Khalid, N. A., Osman, N. & Mansor, N. 2015. Three types of marine microalgae and *Nannocholoropsis oculata* cultivation for potential source of biomass production. *Journal of Physics: Conference Series*, 622, 012034.

[12] Li, Z., Haifeng, L., Zhang, Y., Shanshan, M., Baoming, L., Zhidan, L., Na, D., Minsheng, L., Buchun, S. & Jianwen, L. 2017. Effects of strain, nutrients concentration and inoculum size on microalgae culture for bioenergy from post hydrothermal liquefaction wastewater. *International Journal of Agricultural and Biological Engineering*, 10(2), 194-204.

[13] Lu, S., Wang, J., Ma, Q., Yang, J., Li, X. & Yuan, Y. J. 2013. Phospholipid metabolism in an industry microalga *Chlorella sorokiniana*: The impact of inoculum sizes. *PLoS ONE*, 8(8), e70827.

[14] Ramkumar, S. & Kirubakaran, V. 2016. Biodiesel from vegetable oil as alternate fuel for C.I engine and feasibility study of thermal cracking: A critical review. *Energy Conversion and Management*, 118, 155–169.

[15] Shokravi, Z., Shokravi, H.,Chyuan, O. H., Lau, W. J., Koloor, S. S. R., Petrů, M. & Ismail, A. F. 2020. Improving 'lipid productivity' in microalgae by bilateral enhancement of biomass and lipid contents: A review. *Sustainability*, 12(21), 9083.

[16] Sreedharan, A., Velmurugan, N. & Suresh, A. 2018. Nitrogen replete stress condition for enhanced lipid accumulation in microalgae *Chlorella* sp. *Journal of Algal Biomass Utilization*, 9(1), 37-43.

[17] Wood, A. M., Everroad, R. C. & Wingard, L. M. 2005. Measuring Growth Rates in Microalgal Cultures. *In:* Andersen, R. A. (Ed.). *Algal Culturing Techniques*. Elsevier Academic Press. pp 269-285.

[18] Xu, Y. & Boeing, W. J. 2014. Modeling maximum lipid productivity of microalgae: Review and next step. *Renewable and Sustainable Energy Reviews*, 32, 29-39.

[19] Zarrinmehr, M. J., Farhadian, O., Heyrati, F. P., Keramat, J., Koutra, E., Kornaros, M. & Daneshvar, E. 2020. Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, *Isochrysis galbana*. *The Egyption Journal of Aquatic Research*, 46(2), 153-158.

[20] Zhu, L. D., Li, Z. H. & Hiltunen, E. 2016. Strateries for lipid production improvement in microalgae as a biodiesel feedstock. *BioMed Research International*, 16, 8792548.