

The Effect of NaHCO₃ and Mg²⁺ Addition in *Haematococcus pluvialis* Cultivation by Carbon Injection Method

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Abstract. The emission of carbon dioxide has been continuously rising year by year. Many efforts that have been used with aim of climate recovery, such as capturing CO₂ with the Carbon Capture Storage (CCS) method, which is the CCS technology is one of the effective tactics for reducing carbon emissions by utilizing energy from biomass of microalgae. This research will discuss about carbon capture using microalgae *Haematococcus pluvialis* in a lab scale photobioreactor (PBR), and resulting the optimum biomass productivity for *Haematococcus pluvialis* has occurred in the H₂ variable (50 ppm Mg²⁺). This happens because of addition of Mg ions above 50 ppm can decrease the yield of biomass productivity, since *Haematococcus pluvialis* cannot live in high salinity concentrations. Further research should make a calculation of optimal cost incurred at the optimal carbon concentration that can be captured by microalgae, also the results of increasing value of the microalgae biomass produced for the comprehensive use of the microalgae.

Keywords: CO₂ Capture; Microalgae; *Haematococcus pluvialis*

1. Introduction

Global warming is a never end catastrophic climate change for the earth citizens, and the rise of CO₂ levels in atmosphere is one of the main causes (Atwoli et al., 2021). The emission of CO₂ gas has reached to 412.3 ppm in 2019 (Yoro and Daramola, 2020), and increased to 414.7 ppm in 2021 (Jiang et al., 2022). If the CO₂ levels in atmosphere has reached more than the safety level, it will cause the rise of sea level and also threaten the biodiversity and ecosystem functions (Hilmi et al., 2021). The industrial sector has long been the largest contributor to CO₂ emissions, because of the high energy consuming can put tremendous pressure on the ecological environment (Wang et al., 2021).

To prevail over the carbon gas emissions, one of the solutive way is capturing the carbon. The methods of carbon capture can be divided into four types, such as chemical method using raw materials of polycarbonates, fuel-based method using raw materials of gasoline, conventional method using a solution, and biological exploitation using microalgae for growth source (Vaz Jr. et al., 2022). As an organic source, microalgae have more better benefits for the environment, such as flexibility of medium growth like sea water and wastewater (Ahmad et al., 2021), also having a potential to preclude the problems of global environment as a fossil replacement, this makes microalgae can reduce the problems of energy stocks and leading the sustainability (Subsamram et al., 2019).

Haematococcus pluvialis is one of unicellular microalgae that lives on fresh water and distributed in many habitats around the world. *Haematococcus pluvialis* cells are usually spherical in shape with a diameter of 30 µm. *Haematococcus pluvialis* is considered as the best natural source of astaxanthin and the main producing organism of this commercial product, and most of the applications of Astaxanthin are related to nutrition and human health in the form of food, pharmaceuticals, nutraceuticals, and dietary supplements (Oslan et al., 2021).

Carbon Capture and Storage (CCS) is a process of CO₂ separation to a carbon storage, where CCS aims to reduce carbon emissions and help to achieve the sustainable development goals (Mikunda et al., 2021). Using pipelines, the stored CO₂ gas will later be compressed and stored to the chemical industries such as methanol production, and other resources (Shaw & Mukherjee, 2022). Microalgae could be able to absorb CO₂ as a nutrient and have a carbon concentration mechanism (CCM) that can maximize photosynthetic efficiency under conditions of high and low CO₂ (Prasad et al., 2021). Generally, microalgae production requires well-defined conditions, but the maintenance of large-scale

cultures under outdoor conditions of variable irradiance, temperature, rainfall and other influences presents challenges which are not experienced in the controlled conditions of small-scale laboratory cultures (Masojidek et al., 2021). Closed systems can solve the problems that happen in open systems, because open systems are more prone to contaminants (Mustapa et al., 2020). Choosing a development system of microalgae can affect the bio-fixation process and results of microalgae biomass productivity (Merlo et al., 2021). In most cases, microalgae can be cultivated in an open system such as open pond, or using a closed system such as photobioreactor (PBR) (Chua et al., 2022). Closed photobioreactor system has more advantages because of the more controllable of the environment as well as temperature, intensity of light, and nutrient composition (Assunção and Malcata, 2020). Because of the closed system, this can reduce the possibility of contamination during the cultivation process (Lane, 2021). This research aims to knowing the effect of carbon injection against the growth rate of *Haematococcus pluvialis* and to knowing the effects of adding urea, NaHCO_3 , and Mg^{2+} for the growth rate of microalgae.

2. Method

2.1. Materials

This research use microalgae *Haematococcus pluvialis* as the main sample. The medium culture contains of RO water, 1 N NaOH, NaHCO_3 solution, urea, Mg^{2+} , and CO_2 gas cylinders. The tools used in this study were a series of photobioreactors 120 L, UV-VIS spectrophotometer, gas analyzer, 250 ml beaker glass, 1000 ml volumetric flask, erlenmeyer, evaporating dish, pH meter, conductivity meter, DO meter, cuvette, syringe, and test tube.

2.2. Variables

For the fixed variables, we are using temperature (27-28 °C), nutritional intake for each day of cultivation (10 ml of urea per day and 20 ml of NaHCO_3 per 2 days), and 8 hours of lighting duration. The dependent variable used in this research is the cultivation time is 10 days, pH of microalgae and optical density (OD) for *Haematococcus pluvialis* is 684 nm was measured using a UV-VIS spectrophotometer, both were measured every day. Response variable is pH and OD on each day. For independent variable, we variate the NaHCO_3 as a nutrient (0; 100; and 200 ppm) and variations in Mg^{2+} as a nutrient (0; 50; 75; 100; 125 and 150 ppm).

2.3. Broodstock cultivation and cultivation of *Haematococcus pluvialis*

Cultivation of broodstock was made in two jars with each capacity of 12 L, and the ratio of microalgae and media is 1 to 3. First, prepare a 9 L media solution that contains a mixture of 500 ppm NaHCO_3 and 60 ppm urea. Next, we put 3 L of *Haematococcus pluvialis* microalgae into the media and perform aeration during cultivation. After that, check the OD value during cultivation until you get an OD value greater than 1. After that, prepare a cultivation medium consisting of 500 ml of RO water and mixed with mixture of 60 ppm urea, NaHCO_3 and Mg^{2+} (will be added according to the variables), which have been centrifuged previously. Then, add 1 L of microalgae brooders for each variable and adjust the flow rate of CO_2 gas according to the variable (Figure 1). Microalgae was cultivated for 10 days and observing optical density and pH every day during cultivation, and flowing CO_2 gas every day at a variable flow rate for 120 minutes or until the pH of the microalgae solution is 7.

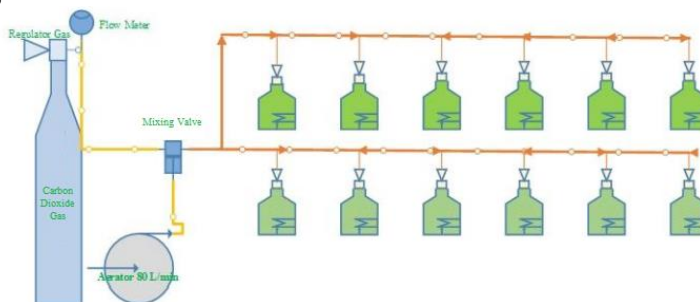


Figure 1. Photobioreactor Schematic in Laboratory-scale

2.4. Microalgae Harvesting

Microalgae harvesting was carried out after microalgae had been cultivated for 10 days. Harvesting was carried out using 0.05 ppm chitosan flocculant and then allowed to stand for 1 day. After that, the microalgae precipitate was

filtered and centrifuged at a speed of 3500 ppm for 10 minutes and then the precipitate was taken. The centrifuged precipitate was dried in an oven at 60°C and checked for 30 minutes once until dry and then weighed.

2.5. Growth rate, Biomass Productivity, CO₂ Fixation Rate, and Dry Biomass Measurement

Growth rate was measured following equation (1), where μ is growth rate per unit time (d⁻¹), C_x is biomass density, and t is time (day). Biomass productivity was measured following equation (2), where Px_{volume} is biomass productivity (g/L.day), and μ is growth rate per unit time (d⁻¹). CO₂ fixation rate was measured following equation (3), where Px_{CO_2} is CO₂ fixation rate (g/L.day) and Px_{volume} is biomass productivity (g/L.day). While dry biomass was measured by following equation (4), where m_{DB} is dry biomass weight (g), m_{ED} is weight of evaporating dish (g), and m_{WB} is wet biomass weight (g).

$$\mu = \frac{\ln\left(\frac{C_{x2}}{C_{x1}}\right)}{t_2 - t_1} \quad (1)$$

$$Px_{volume} = \mu \times C_x \quad (2)$$

$$Px_{CO_2} = 1.83 \times Px_{volume} \quad (3)$$

$$m_{DB} = (m_{ED} + m_{WB}) - m_{ED} \quad (4)$$

3. Result and Discussion

3.1. Optical Density of *Haematococcus pluvialis*

Optical density measurement has a purpose in the world of microalgae cultivation, besides of obtaining the quantity of microalgae or biomass for each variable, and also used to determine microalgae concentration (Nunes et al., 2021), optical density measurement can re-validate the data obtained in order to determine more precisely growth phase (Rinawati and Prasetyo, 2020). Measurement of optical density using a UV-VIS spectrophotometer with a wavelength for the microalgae *Haematococcus pluvialis* is 684 nm. From the measurements, the optical density versus time curve obtained as shown in the Figure 2a-c. The main purpose of NaHCO₃ and Mg²⁺ in a growth medium of microalgae cultivation is for supplying nutrients that used in photosynthesis during microalgae growth. Urea as a nitrogen source, NaHCO₃ as a carbon source, and Mg²⁺ as a macronutrient which is an absolute essential element (Polat et al., 2020). The OD value will increase concomitantly with the increase of microalgae density, which means that every increase in the absorbance value (OD) will be followed by the number of microalgae, where the OD value also shows the biomass contained in microalgae (Nielsen & Hansen, 2019).

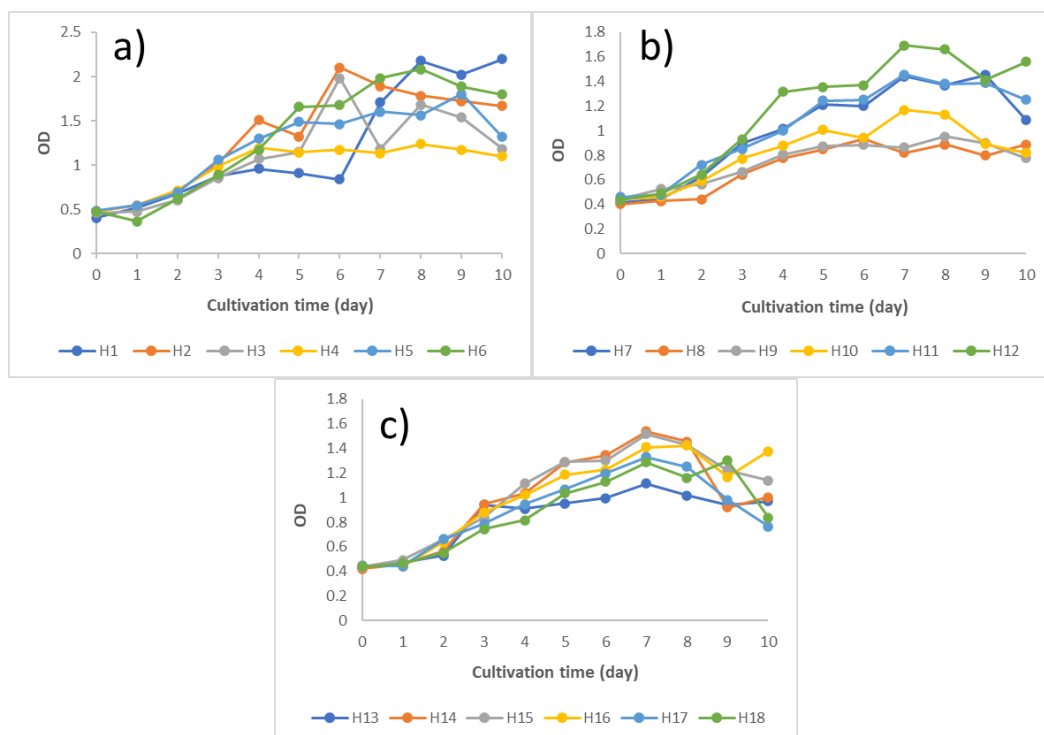


Figure 2. Optical density of *Haematococcus pluvialis* with NaHCO₃ a) 0, b) 100, and c) 200 ppm

3.2. Growth Rate of *Haematococcus pluvialis*

The growth rate of microalgae was obtained by calculating the ln ratio of the biomass concentration when it reached the exponential phase to the biomass concentration at the start of cultivation divided by the time needed during the exponential phase (Pourjamshidian et al., 2019). From these calculations, the growth rate versus cultivation time curve of microalgae *Haematococcus pluvialis* obtained as shown in the Figure 3a-c. Based on the graph, microalgae *Haematococcus pluvialis* entered the exponential phase on day 2 of cultivation. Lag phase occurs the introduction of inoculum into the culture medium, delays the rate of growth because microalgae adapting to the new medium before cell division occurred. Exponential phase occurs the main process of microalgae cultivation, which means there is an increase of biomass. Structure of cells has already in the normal conditions, also the nutrients in the media and the nutrient content in the cells has been balanced. Microalgae will enter the death phase, which is indicated by a decrease in the number of cells. Death phase was indicated by the decreasing of nutrient content in the cultivation media and the decreased metabolic ability of microalgae due to age factor (Yousuf, 2020).

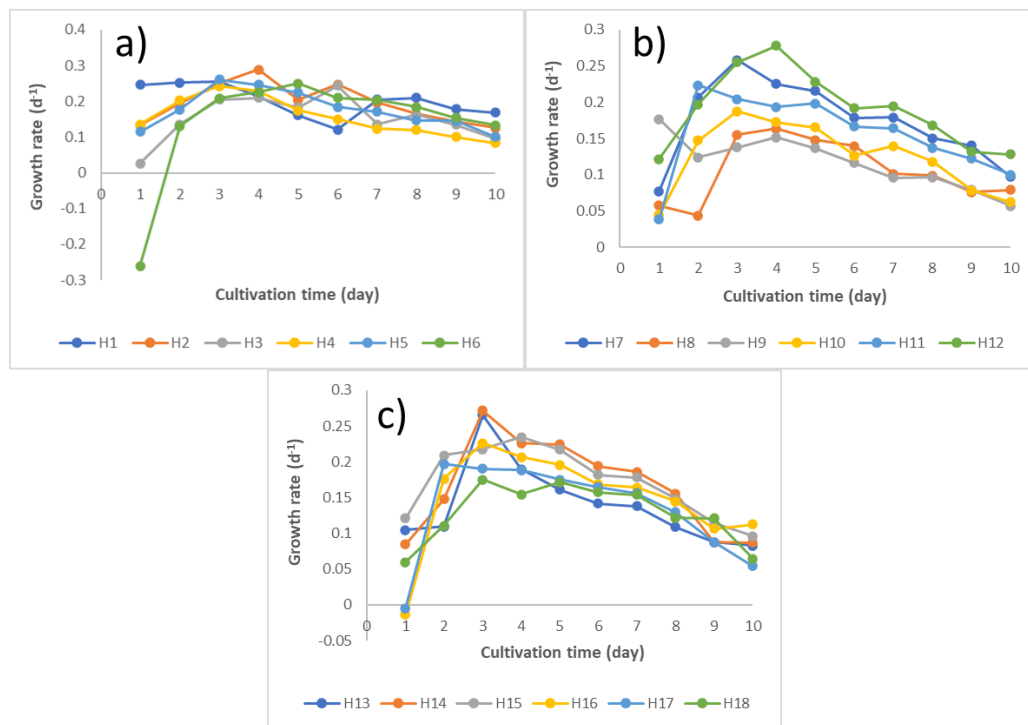


Figure 3. Growth rate of *Haematococcus pluvialis* with NaHCO_3 a) 0, b) 100, and c) 200 ppm

3.3. Biomass Productivity of *Haematococcus pluvialis*

The results of biomass productivity in this study were obtained by calculating the growth rate per unit time (d^{-1}) multiplied by the biomass or cell density result (Lucáková et al., 2022). From these calculations, the biomass productivity versus cultivation time curve of microalgae *Haematococcus pluvialis* obtained as shown in the Figure 4a-c. Based on graph, the optimal biomass productivity of *Haematococcus pluvialis* for variable NaHCO_3 0 ppm is 0.52 g/L.day in day 6 with addition of urea 60 ppm and Mg^{2+} 50 ppm, for variable NaHCO_3 100 ppm is 0.266 g/L.day in day 4 with addition of urea 60 ppm and Mg^{2+} 150 ppm, and for variable NaHCO_3 200 ppm is 0.288 g/L.day in day 5 with addition of urea 60 ppm and Mg^{2+} 50 ppm. The results of biomass productivity in cultivation with higher CO_2 capture are influenced by carbon concentrating mechanism factors, using CA (Carbonic Anhydrase) found in intracellular and extracellular. The function of CA is to support the photosynthetic process of carbonate compounds into biomass. Carbon units that contained in the culture medium will become saturated, and will turn into carbonate compounds when reacted with water. This carbonate compound will be converted into biomass with the help of CA (Wang and Wei, 2020).

The graph on figure above shows a negative values of biomass productivity on the variable H6, H16, and H17. This is because the OD value on day 1 is smaller than the OD value on day 0 because there are impurities in the microalgae. The decrease in OD value on day 1 was also due to the microalgae entering the lag phase where the microalgae conditions were adapting to the given nutrients.

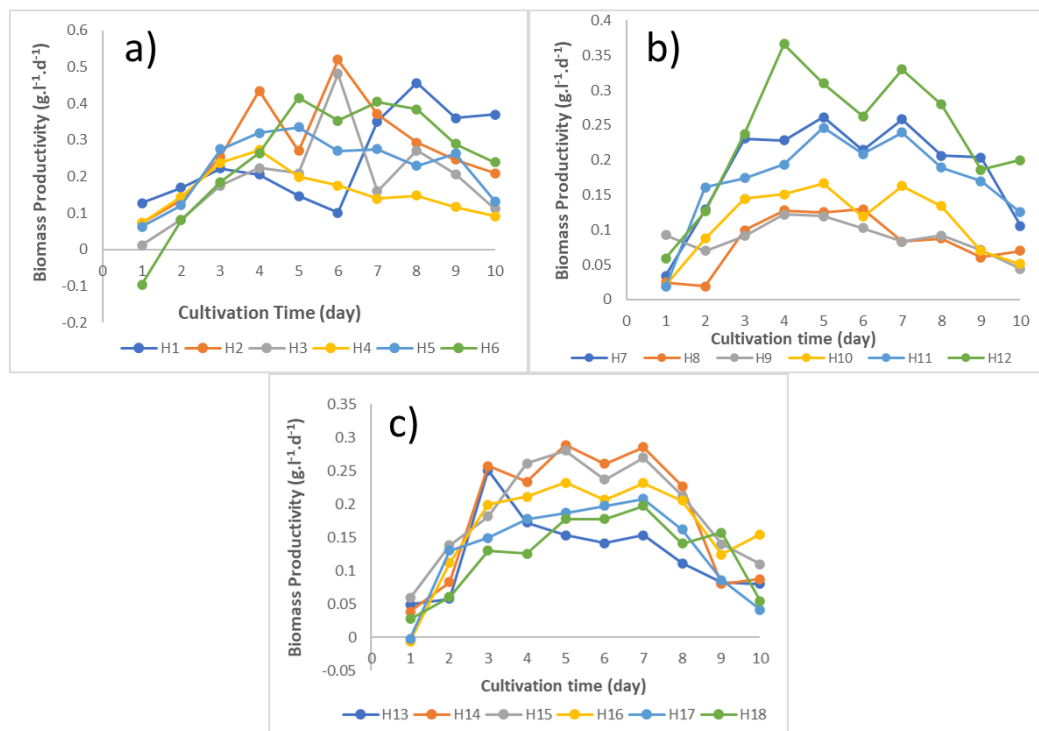


Figure 4. Biomass productivity of *Haematococcus pluvialis* with NaHCO_3 a) 0, b) 100, and c) 200 ppm

The addition of Mg^{2+} ions is expected to be a growth promoter which encourages algae photosynthesis by accelerating the conversion of CO_2 into bicarbonate substrate. The addition of MgAC (Magnesium aminoclay) to microalgae cultivation with a concentration of 50 ppm had optimal biomass productivity by controlling temperature, light intensity and pH 9 (Kim et al., 2020). The addition of MgAC above 50 ppm caused a decrease in biomass productivity, due to *Haematococcus pluvialis* could not live in high salinity concentrations. This causes a decrease in the productivity of microalgae biomass at the addition of 150 ppm Mg^{2+} in the H₁₂ variable. The nutrient NaHCO_3 serves as a carbon source for microalgae during cultivation. However, when CO_2 flows through microalgae, microalgae have the potential to become saturated during cultivation because they absorb too much carbon, which causes a decrease in the productivity of microalgae biomass.

3.4. CO_2 Fixation Rate of *Haematococcus pluvialis*

The results of CO_2 fixation rate in this study were obtained from the yield of biomass productivity multiplied by 1.83 (Pires da Mata Costa et al., 2021). From these calculations, the CO_2 fixation rate versus cultivation time curve of microalgae *Haematococcus pluvialis* obtained as shown in the Figure 5a-c. When we cultivate microalgae with NaHCO_3 nutrient and addition of CO_2 , the carbon source in microalgae biomass comes from HCO_3^- and CO_2 . As for the ideal carbon capture system, microalgae absorb more carbon sources from CO_2 than from NaHCO_3 medium (Kassim et al., 2020). Therefore, the efficiency of CO_2 fixation will increase as the bicarbonate concentration decreases (Zhu et al., 2020)

Based on the graph, the optimal CO_2 fixation efficiency for microalgae *Haematococcus pluvialis* occurred in the H₂ variable with variations of 60 ppm urea, 50 ppm Mg^{2+} , and 0 ppm NaHCO_3 . While the addition of 100 ppm and 200 ppm NaHCO_3 respectively is the H₁₂ variable with variations of 60 ppm urea, 150 ppm Mg^{2+} and the H₁₄ variable with variations of 60 ppm urea, 50 ppm Mg^{2+} . The results of this study concludes when the higher the concentration of NaHCO_3 in microalgae culture, the efficiency of CO_2 fixation will decrease.

3.5. Correlation Between Variables and Dry Biomass of *Haematococcus pluvialis*

The results of dry biomass of *Haematococcus pluvialis* in this study are shown in the Figure 6. When we harvesting the microalgae, 0.05 grams of chitosan flocculant was added. Harvesting microalgae cells by flocculation is considered better than conventional methods such as centrifugation or filtration because it can produce better biomass in quantity (Shaikh et al., 2020). The addition of the macronutrient Mg^{2+} is an absolute essential element that must be available even in small amounts (Ermis et al., 2020). Magnesium metal cation is the core of the chlorophyll molecule which is absolutely needed by microalgae to increase chlorophyll production, but can have a negative impact on microalgae growth in conditions of Mg^{2+} deficiency or surplus media, so that the right concentration is needed (Liu et al., 2022). Based on the

research results obtained, the optimum dry biomass production was produced by the H₂ variable, namely 0.728 grams with treatment of 0 ppm NaHCO₃, 60 ppm urea, and 50 ppm Mg²⁺. Optimum biomass productivity is also produced by the H₂ variable. This is because the higher the biomass productivity, the higher the dry biomass produced (de Souza et al., 2022).

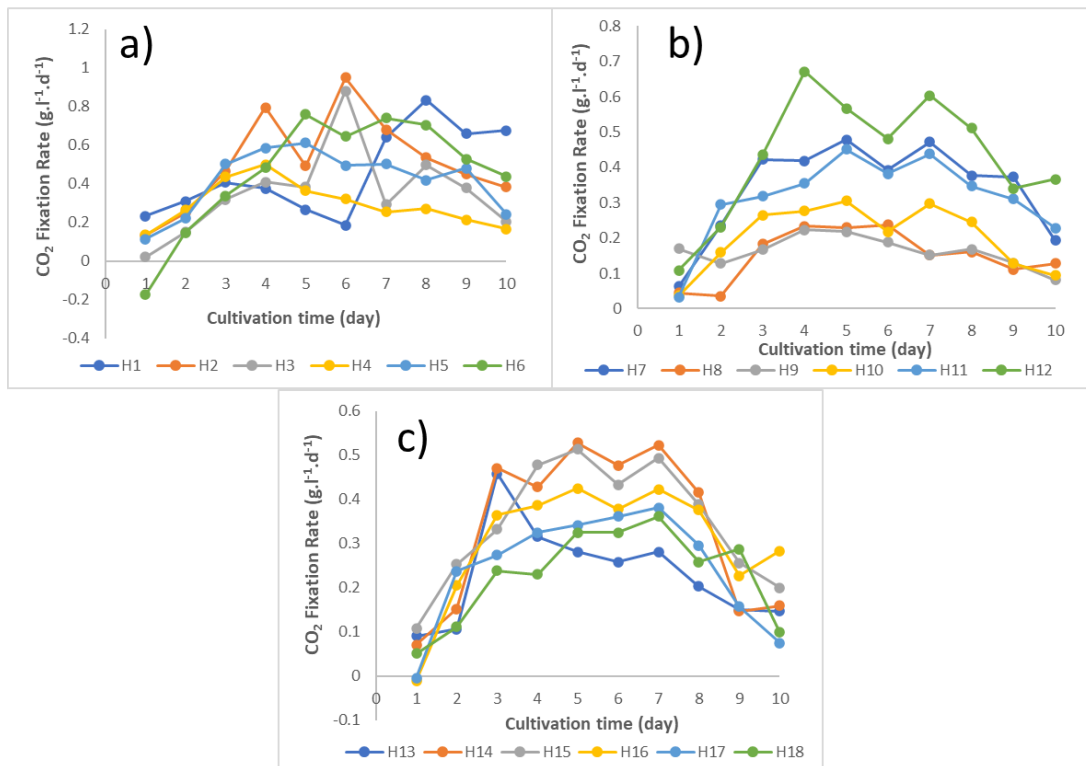


Figure 5. CO₂ fixation rate of *Haematococcus pluvialis* with NaHCO₃ a) 0, b) 100, and c) 200 ppm

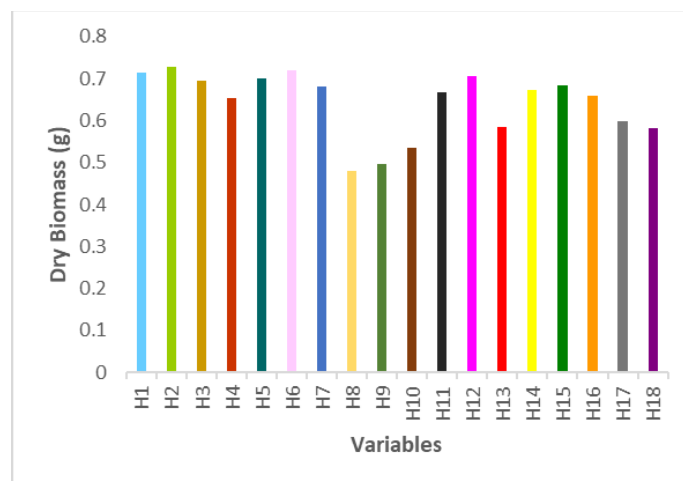


Figure 6. Dry biomass of *Haematococcus pluvialis* for each variable

4. Conclusion

The results of this study concludes that every increase in the absorbance value (OD) will be followed by the number of microalgae *Haematococcus pluvialis*. When the higher the concentration of NaHCO₃ and flow rate of CO₂ in microalgae culture, it will decrease the growth rate, biomass productivity, and also the efficiency of CO₂ fixation. The higher

of biomass productivity will also produce the higher yield of dry biomass. Suggestions given for further research, the volume of microalgae should be maintained during cultivation, keeping the aerator from being too tight, double check the equipment periodically to ensure that the tool settings are in the same condition during cultivation, and control the conditions that need to be considered to produce optimum biomass.

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References

- Ahmad, I., Yuzir, A., Mohamad, S. E., Iwamoto, K., & Abdullah, N. (2021). Role of microalgae in sustainable energy and environment. *IOP Conference Series: Materials Science and Engineering*, 1051(1), 012059. <https://doi.org/10.1088/1757-899X/1051/1/012059>
- Assunção, J., & Malcata, F. X. (2020). Enclosed “non-conventional” photobioreactors for microalga production: A review. *Algal Research*, 52, 102107. <https://doi.org/10.1016/j.algal.2020.102107>
- Atwoli, L., Baqui, A. H., Benfield, T., Bosurgi, R., Godlee, F., Hancocks, S., Horton, R., Laybourn-Langton, L., Monteiro, C. A., Norman, I., Patrick, K., Praities, N., Rikkert, M. G. M. O., Rubin, E. J., Sahni, P., Smith, R., Talley, N., Turale, S., & Vázquez, D. (2021). Call for emergency action to limit global temperature increases, restore biodiversity, and protect health: Wealthy nations must do much more, much faster. *Nutrition Reviews*, 79(11), 1183-1185. <https://doi.org/10.1093/nutrit/nuab067>
- Chua, S. Y., Cheng, Y. W., Lam, M. K., Dasan, Y. K., Kadir, W. N. A., Rosli, S. S., Lim, J. W., & Lim, S. (2022). *Microalgae cultivation for sustainable biofuel production*. In Value-chain of biofuels (pp. 137-158). Elsevier. <https://doi.org/10.1016/B978-0-12-824388-6.00006-3>
- de Souza, D. S., Valadão, R. C., de Souza, E. R. P., Barbosa, M. I. M. J., & de Mendonça, H. V. (2022). Enhanced *Arthrospira platensis* biomass production combined with anaerobic cattle wastewater bioremediation. *Bioenergy research*, 15(1), 412-425. <https://doi.org/10.1007/s12155-021-10258-4>
- Ermis, H., Guven-Gulhan, U., Cakir, T., & Altinbas, M. (2020). Effect of iron and magnesium addition on population dynamics and high value product of microalgae grown in anaerobic liquid digestate. *Scientific reports*, 10(1), 1-12. <https://doi.org/10.1038/s41598-020-60622-1>
- Hilmi, N., Chami, R., Sutherland, M. D., Hall-Spencer, J. M., Lebleu, L., Benitez, M. B., & Levin, L. A. (2021). The role of blue carbon in climate change mitigation and carbon stock conservation. *Frontiers in Climate*, 102. <https://doi.org/10.3389/fclim.2021.710546>
- Jiang, F., Ju, W., He, W., Wu, M., Wang, H., Wang, J., ... & Chen, J. M. (2022). A 10-year global monthly averaged terrestrial net ecosystem exchange dataset inferred from the ACOS GOSAT v9 XCO₂ retrievals (GCAS2021). *Earth System Science Data*, 14(7), 3013-3037. <https://doi.org/10.5281/zenodo.5829774>
- Kassim, M. A., Adnan, M. F. I. M., Tan, K. M., Bakar, M. H. A., Lalung, J., & Mohamed, M. S. (2020). Carbonic anhydrase (CA) activity by *Chlorella* sp. in immobilised matrix under carbon dioxide rich cultivation condition. In *IOP Conference Series: Materials Science and Engineering*, 716(1), 012015. <https://doi.org/10.1088/1757-899X/716/1/012015>
- Kim, Y. E., Matter, I. A., Lee, N., Jung, M., Lee, Y. C., Choi, S. A., Lee, S. Y., Kim, J. R., & Oh, Y. K. (2020). Enhancement of astaxanthin production by *Haematococcus pluvialis* using magnesium aminoclay nanoparticles. *Bioresource technology*, 307, 123270. <https://doi.org/10.1016/j.biortech.2020.123270>
- Lane, T. W. (2022). Barriers to microalgal mass cultivation. *Current Opinion in Biotechnology*, 73, 323-328. <https://doi.org/10.1016/j.copbio.2021.09.013>
- Liu, S. Y., Zhao, R. Z., Qiu, X. C., & Guo, Q. (2022). Optimization Analysis to Evaluate the Relationships between Different Ion Concentrations and *Prymnesium parvum* Growth Rate. *Water*, 14(6), 928. <https://doi.org/10.3390/w14060928>

- Lucakova, S., Branyikova, I., Branyik, T., Matoulkova, D., & Krausova, G. (2022). Wastewater from the demineralization of cheese whey for cost-efficient cultivation of spirulina. *Journal of Applied Phycology*, 34(1), 89-99. <https://doi.org/10.1007/s10811-021-02644-4>
- Masojídek, J., Ranglová, K., Lakatos, G. E., Silva Benavides, A. M., & Torzillo, G. (2021). Variables governing photosynthesis and growth in microalgae mass cultures. *Processes*, 9(5), 820. <https://doi.org/10.3390/pr9050820>
- Merlo, S., Gabarrell Durany, X., Pedroso Tonon, A., & Rossi, S. (2021). Marine microalgae contribution to sustainable development. *Water*, 13(10), 1373. <https://doi.org/10.3390/w13101373>
- Mikunda, T., Brunner, L., Skylogianni, E., Monteiro, J., Rycroft, L., & Kemper, J. (2021). Carbon capture and storage and the sustainable development goals. *International Journal of Greenhouse Gas Control*, 108, 103318. <https://doi.org/10.1016/j.ijggc.2021.103318>
- Mustapa, N. S., Mansor, M. A., & Serri, N. A. (2020). Design and development of centred-light photobioreactor for microalgae cultivation system. *IOP Conference Series: Materials Science and Engineering*, 716(1), 012009. <https://doi.org/10.1088/1757-899X/716/1/012009>
- Nielsen, S. L., & Hansen, B. W. (2019). Evaluation of the robustness of optical density as a tool for estimation of biomass in microalgal cultivation: The effects of growth conditions and physiological state. *Aquaculture research*, 50(9), 2698-2706. <https://doi.org/10.1111/are.14227>
- Nunes, N. S. P., Oliveira, I. G. S., Ansilago, M., & de Carvalho, E. M. (2021). Analysis of functional relationships between the cell quantification variables in the microalgae *Chlorella sorokiniana*. *Revista Ibero-Americana de Ciências Ambientais*, 12(1), 453-460. <https://doi.org/10.6008/CBPC2179-6858.2021.001.0036>
- Oslan, S. N. H., Shoparwe, N. F., Yusoff, A. H., Rahim, A. A., Chang, C. S., Tan, J. S., ... & Mohamed, M. S. (2021). A review on *Haematococcus pluvialis* bioprocess optimization of green and red stage culture conditions for the production of natural astaxanthin. *Biomolecules*, 11(2), 256. <https://doi.org/10.3390/biom11020256>
- Pires da Mata Costa, L., Micheline Vaz de Miranda, D., Couto de Oliveira, A. C., Falcon, L., Stella Silva Pimenta, M., Guilherme Bessa, I., Wouters, S. J., Andrade, M. H. S., & Pinto, J. C. (2021). Capture and reuse of carbon dioxide (CO₂) for a plastics circular economy: A review. *Processes*, 9(5), 759. <https://doi.org/10.3390/pr9050759>
- Polat, E., Yüksel, E., & Altınbaş, M. (2020). Mutual effect of sodium and magnesium on the cultivation of microalgae *Auxenochlorella protothecoides*. *Biomass and bioenergy*, 132, 105441. <https://doi.org/10.1016/j.biombioe.2019.105441>
- Pourjamshidian, R., Abolghasemi, H., Esmaili, M., Amrei, H. D., Parsa, M., & Rezaei, S. (2019). Carbon dioxide biofixation by *Chlorella* sp. in a bubble column reactor at different flow rates and CO₂ concentrations. *Brazilian Journal of Chemical Engineering*, 36, 639-645. <https://doi.org/10.1590/0104-6632.20190362s20180151>
- Prasad, R., Gupta, S. K., Shabnam, N., Oliveira, C. Y. B., Nema, A. K., Ansari, F. A., & Bux, F. (2021). Role of microalgae in global CO₂ sequestration: physiological mechanism, Recent Development, Challenges, and Future Prospective. *Sustainability*, 13(23), 13061. <https://doi.org/10.3390/su132313061>
- Rinawati, M., Sari, L. A., & Pursetyo, K. T. (2020). Chlorophyll and carotenoids analysis spectrophotometer using method on microalgae. *IOP Conference Series: Earth and Environmental Science*, 441(1), 012056. <https://doi.org/10.1088/1755-1315/441/1/012056>
- Shaikh, S. M., Hassan, M. K., Nasser, M. S., Sayadi, S., Ayesh, A. I., & Vasagar, V. (2021). A comprehensive review on harvesting of microalgae using Polyacrylamide-Based Flocculants: Potentials and challenges. *Separation and Purification Technology*, 277, 119508. <https://doi.org/10.1016/j.seppur.2021.119508>
- Shaw, R., & Mukherjee, S. (2022). The development of carbon capture and storage (CCS) in India: A critical review. *Carbon Capture Science & Technology*, 2, 100036. <https://doi.org/10.1016/j.ccst.2022.100036>
- Subsamran, K., Mahakhan, P., Vichitphan, K., Vichitphan, S., & Sawaengkaew, J. (2019). Potential use of vetiver grass for cellulolytic enzyme production and bioethanol production. *Biocatalysis and Agricultural Biotechnology*, 17, 261-268. <https://doi.org/10.1016/j.bcab.2018.11.023>
- Vaz Jr, S., de Souza, A. P. R., & Baeta, B. E. L. (2022). Technologies for carbon dioxide capture: A review applied to energy sectors. *Cleaner Engineering and Technology*, 8, 100456. <https://doi.org/10.1016/j.clet.2022.100456>

- Wang, J., Sun, K., Ni, J., & Xie, D. (2021). Evaluation and Factor Analysis of Industrial Carbon Emission Efficiency Based on “Green-Technology Efficiency”—The Case of Yangtze River Basin, China. *Land*, 10(12), 1408. <https://doi.org/10.3390/land10121408>
- Wang, Q., Yu, Z., & Wei, D. (2020). High-yield production of biomass, protein and pigments by mixotrophic *Chlorella pyrenoidosa* through the bioconversion of high ammonium in wastewater. *Bioresource Technology*, 313, 123499. <https://doi.org/10.1016/j.biortech.2020.123499>
- Yoro, K. O., & Daramola, M. O. (2020). CO₂ emission sources, greenhouse gases, and the global warming effect. In *Advances in carbon capture* (pp. 3-28). Woodhead Publishing. <https://doi.org/10.1016/B978-0-12-819657-1.00001-3>
- Yousuf, A. (2020). *Fundamentals of microalgae cultivation*. In *Microalgae cultivation for biofuels production* (pp. 1-9). Academic Press. <https://doi.org/10.1016/B978-0-12-817536-1.00001-1>
- Zhu, C., Zhai, X., Xi, Y., Wang, J., Kong, F., Zhao, Y., & Chi, Z. (2020). Efficient CO₂ capture from the air for high microalgal biomass production by a bicarbonate Pool. *Journal of CO₂ Utilization*, 37, 320-327. <https://doi.org/10.1016/j.jcou.2019.12.023>