

Research Article

Effects of different culture media on growth and lutein production in *Tetradesmus dimorphus* NUAC202

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Abstract

Microalgae are a source of valuable compounds with broad applications in cosmeceuticals, nutraceuticals, renewable energy, and bioremediation. The present study employs the freshwater microalgae *Tetradesmus dimorphus* NUAC202 to investigate the effects of different microalgal growth media, like BG-11, Bold's basal medium (BBM), Bold's basal medium with 3-fold nitrogen (3N-BBM), Modified Mann & Myers medium (MMM), Modified Bristol's medium (BMM) and Modified Detmer's medium (MDM), on its growth, biochemical compounds and photosynthetic pigment production. Lutein and β -carotene content were measured by using high-performance liquid chromatography (HPLC). Growth parameters were significantly improved by MDM and BMM media, whereas maximum accumulation of pigments, especially lutein (2.68 mg/g DW) and β -carotene (0.76 mg/g DW) were observed in BG-11 medium. Moreover, MDM and BG-11-biomass achieved high protein yield (40.64% and 40.49%, respectively). Thus, this study proposes MDM and BG-11 as a suitable media to cultivate *T. dimorphus* NUAC202 for the co-production of lutein and protein.

Keywords: *Tetradesmus dimorphus*, Culture medium, Lutein, β -carotene, Biochemical compounds

Introduction

Carotenoids possess notable antioxidant properties, enabling their widespread use in healthcare products, food colorants, and feed additives (Dufossé, 2018; Sathasivam & Ki, 2018). Among the various carotenoids found in nature, lutein is particularly valuable due to its potential to enhance cardiovascular health, combat certain types of cancer, and address human degenerative diseases (Asker *et al.*, 2018; Sathasivam *et al.*, 2019). Lutein plays a significant role in photosynthesis and belongs to the class of xanthophyll and provides photoprotection to microalgae. Recognized as a food additive (E161b) approved by the European Union, lutein has a market value exceeding \$150 million in the US alone (Cérón *et al.*, 2008). Currently, marigold oleoresin (*Tagetes erecta*) is the primary commercial source for lutein production, but challenges such as low lutein content (around 0.03%) and limited biomass yield hinder large-scale application (Piccaglia *et al.*, 1998).

Microalgae are fast-growing photoautotrophs with the capability to serve as energy feedstocks and sources of various high-value products, including chemicals, pigments, polysaccharides, and vitamins (Chang *et al.*, 2015; Lin *et al.*, 2015). Changes in physical parameters—such as light intensity, photoperiod, temperature, and pH—or chemical factors like nutrients (nitrogen, phosphorus, salinity) expose microalgae to stress conditions, which stimulate biomass accumulation and production of valuable biomolecules (Procházková *et al.*, 2014). Species such as *Chlorella protothecoides*, *Chlorella zofingiensis*, *Muriellopsis* sp.,

and *Tetradesmus* sp. are promising candidates for lutein production due to their higher lutein content, faster growth rates and shorter doubling times. Nitrogen starvation is a well-known method to enhance biochemical parameters such as carbohydrate and lipid content. Ördög *et al.* (2012) found that moderate nitrogen starvation yields higher biomass and lipid production compared to complete nitrogen limitation.

T. dimorphus NUAC202 contains approximately 40-50% lutein of the total carotenoid content and is a fast-growing microalga, which led to its selection for this study. The objective of this study is to optimize a suitable growth media for *T. dimorphus* NUAC202 for enhancing the growth, biomass, biochemical composition (protein and carbohydrate content), and carotenoid profile (lutein and β -carotene). The relative amounts of lutein and β -carotene were analyzed using high-performance liquid chromatography (HPLC). Additionally, the study included the assessment of biochemical analyses during the cultivation process.

Materials and methods

Microalgae and culture conditions

Tetradesmus dimorphus (strain NUAC202) used in this study was obtained from the microalgae bank of Fisheries Science Program, Faculty of Agriculture Natural Resources and Environment, Naresuan University. The microalgal cells were initially pre-cultured in Erlenmeyer flasks containing 1 L of BG-11 medium at 25±1 °C with 0.5 L microalgal cultures. Then, the cultures were cultivated under the cool

white, fluorescent lamps (100 $\mu\text{mol photons/m}^2/\text{s}$) for 14 Light (L)/10 Dark (D) photoperiod. The microalgal cultures were manually shaken 2 times per day. When the culture reached the stationary phase, microalgal cells were used for experiments.

Experimental sections

This experiment aims to determine the most suitable growth medium to maximize biomass production and promote high co-production of lutein and protein. The microalga *T. dimorphus* NUAC202 was cultivated in six different autotrophic growth media to determine the most favorable medium for high biomass, lutein, and protein content. Optimizing a suitable growth medium is essential to estimate the productivity of desired products from this strain. Media selection was based on nutrient composition and the concentration of key nutrients necessary for microalgal growth. All experiments were performed in triplicate using 500 mL sterilized Erlenmeyer flasks, each containing 300 mL of sterile autotrophic media such as BG-11, BBM, 3N-BBM, MMM, BMM and MDM media. The flasks were inoculated with 10% of a fully grown (stationary phase) culture of the microalga. The batch culture of *T. dimorphus* NUAC202 was incubated for 12 days and grown photoautotrophically in an artificial culture room maintained at 25 ± 1 °C with continuous light, under a light intensity of 120 $\mu\text{mol photons/m}^2/\text{s}$. The cultures were aerated with air supplemented with 3% (v/v) CO_2 and the experimental flasks were shaken twice daily. To estimate the growth parameters of the microalga, samples from cultures in various growth media were taken every second day throughout the study.

Determination of cell density, biomass, content of photosynthetic pigments

Cell density was determined using a hemocytometer. For biomass measurements, 10 mL of algal samples were centrifuged and then dried at 105 °C until a constant weight was achieved. A total of 2 mL of algal culture was centrifuged at 10,000 rpm at 4 °C for 4 min. Pigment were extracted by adding 2mL of DMSO to the cell pellet. The chlorophyll a and total carotenoid contents present in the supernatants were quantified at 480, 649.1 and 665 nm wavelength by spectrophotometer (V-5100, China), followed by calculation according to Liu (2018).

Determination of lutein and β -carotene

Total lutein and β -carotene were extracted and determined following Sanpapao *et al.* (2023). The composition of pigment profiles extracted from algal biomass were analyzed using HPLC with diode array detection (DAD) (Sher *et al.*, 2018), containing C30 YMC carotenoid column (5 μm , 250 X 4.6 mm I.D). Standards of β -carotene obtained from Sigma-Aldrich, Inc. (Merck KGaA, Darmstadt, Germany) and lutein standard was from ChromaDex (ChromaDex, Irvine, United States).

Determination of protein and carbohydrate content

Total carbohydrate content was estimated by the process according to Kochart (1978) after hydrolysis in 2M H_2SO_4 at 100 °C for 2 h, whereas the glucose (VWR

International Ltd., England) used a standard. Protein content was analyzed by the process according to Lowry *et al.* (1951), using bovine serum albumin (BSA) as a standard.

Statistical analysis

All data was analyzed by one-way ANOVA analysis using R version 4.5.2 (R Development Core Team, 2025). All significant levels were set at $P < 0.05$.

Results and discussion

Effect of different culture media on the growth and biomass of *T. dimorphus* NUAC202

Researchers have investigated various biochemical and genetic approaches to increase the production of target microalgal metabolites. In addition to biochemical and environmental factors influencing microalgal yield, media composition is a key determinant of growth and metabolic activity. Therefore, this study assessed the growth variation of *T. dimorphus* NUAC202 in six widely used culture media—BG-11, BBM, 3N-BBM, MMM, BMM and MDM. The growth of this microalga was measured every two days over a period of 12 days by recording absorbance at 720 nm using a spectrophotometer, as illustrated in Figure 1. The growth curve of *T. dimorphus* NUAC202 showed that the highest growth was achieved in MDM and BMM media than that of the other medias (3N-BBM, BBM, and MMM, BG-11 medium). Additionally, the biomass of *T. dimorphus* NUAC202 exhibits a similar trend in both MDM and BMM

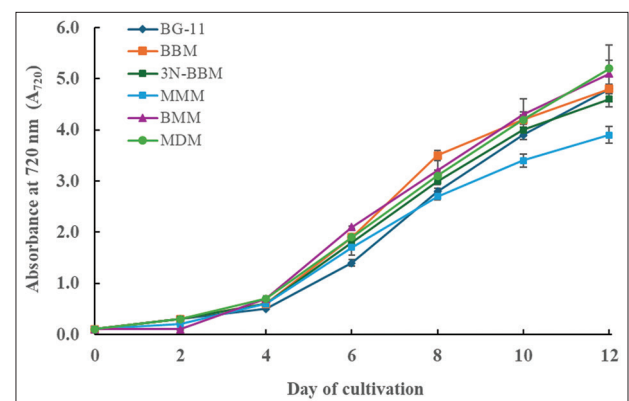


Figure 1: Effect of culture media on *T. dimorphus* NUAC202 growth

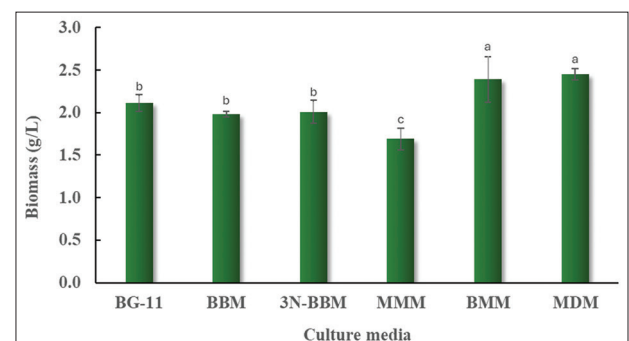


Figure 2: Effect of culture media on *T. dimorphus* NUAC202 biomass

media as shown in Figure 2. The highest biomass was observed in MDM medium (2.45 g/L) followed by 2.39, 2.11, 2.01, 1.98 and 1.69 g/L for BMM, BG11, 3N-BBM, BBM and MMM media, respectively. This may be attributed to differences in nutrient components, particularly the ratios of nitrate and phosphates, which are key macronutrients that significantly influence the biomass productivity of microalgae (Mandalam & Palsson, 1998). The reason for the highest biomass of *T. dimorphus* NUAC202 might be due to that the MDM medium composition has higher concentration of both nitrate (1.0 g/L) and phosphorus (0.25 g/L). Nitrate and phosphorus improve the microalga metabolism, which enhance the cell division. The reason for the lowest biomass in MMM is the limitation of phosphorus, resulting in prevention of metabolic activities which lead to low biomass content. Hong and Lee (2008) reported that KH_2PO_4 and K_2HPO_4 are important for microalgae growth, as they provide the phosphorus necessary for synthesizing DNA, RNA, and the energy molecule ATP. The content of phosphorus is present in lower amount in MDM (0.25 g/L), BMM (0.25 g/L), 3N-BBM (0.25 g/L), BBM (0.25 g/L), however it is present in a trace amount in MMM (0.1 g/L) and BG-11 (0.04 g/L). For quantity of nitrate content is present in BG-11 (1.5 g/L), MDM (1.0 g/L), MMM (1.0 g/L), BMM (0.75 g/L), 3N-BBM (0.75 g/L) and BBM (0.25 g/L).

Effect of media composition on the photosynthetic pigments of *T. dimorphus* NUAC202

The highest chlorophyll *a* content was achieved in the cells grown in MDM medium (37.72 µg/mL) followed by BG-11 (33.53 µg/mL), BMM (32.58 µg/mL), 3N-BBM (28.97 µg/mL) and MMM medium (25.24 µg/mL) (Figure 3a). The pigment level was lowest in the cells grown in BBM (12.84 µg/mL), which shows that the lower photosynthetic activity was achieved in the microalga cell. Overall, these results demonstrate that the chlorophyll levels were significantly influenced by the different growth media. The pigments content might be influenced by the variation in the chemical composition and concentrations of growth medium. Among six medium, BG-11 and MDM showed it is most suitable media for the growth and enhance the photosynthetic pigments in *T. dimorphus* NUAC202. Similar results were reported in *Ankistrodesmus falcatus* (George *et al.*, 2014), *Acutodesmus dimorphus* (Chokshi *et al.*, 2016) and *Scenedesmus rotundus*-MG910488 (Dixit *et*

al., 2022), where the growth and photosynthetic pigment content increased when culturing the cells under BG-11 medium.

Carotenoids are essential pigments that safeguard chlorophyll from photodamage by redirecting excess light away from the reaction centers (Sathasivam *et al.*, 2021). Previous studies have reported that increasing the production of valuable compounds like carotenoids through nutritional or environmental stresses is an effective approach (Bermejo *et al.*, 2018; Lee *et al.*, 2018). The highest carotenoid content was achieved in the cells grown in MDM and BG-11 medium (15.72 and 15.02 µg/mL, respectively), whereas the lowest amounts of carotenoid were found in BBM (6.21 µg/mL) (Figure 3b). Moreover, HPLC result showed that the highest carotenoid profile contents, i.e., lutein and β-carotene, were observed in the cells grown in BG-11 medium (2.68 mg/g DW and 0.76 mg/g DW, respectively) followed by BMM (2.61 mg/g DW and 0.60 mg/g DW, respectively) and MDM medium (2.34 mg/g DW and 0.56 mg/g DW, respectively), whereas the lowest amounts was found in BBM (1.21 mg/g DW and 0.18 mg/g DW, respectively) (Table 1). Similarly, the highest lutein content was found BG11, BMM, and MDM medium, which might be due to the presence of higher nitrate concentrations in these media during cultivation, compared to BBM medium. Furthermore, the color of algae cultures changed gradually according to different nitrate concentrations. Under BBM medium cultivation, the cell color is light green at the earlier stage, whereas on the 10th day it changed to yellowish green. Nitrogen is a key factor for lutein accumulation, and lutein levels decline when nitrogen becomes limited (Xie *et al.*, 2013). Lutein is a primary carotenoid, and it is considered as a growth-coupled metabolite, optimal growth and balanced nutrient conditions enhance the lutein content in microalgae (Gong & Bassi, 2016).

Effect of media composition on biochemical compounds of *T. dimorphus* NUAC202

Apart from the optimal environmental conditions, the macro- and micro-nutrient composition of the media also plays a crucial role in microalgal growth and regulating metabolic pathways (Sterner & Grover, 1998). In the present study, microalgal biomass obtained using MDM and BG-11 medium showed the highest protein content, however in MMM, 3N-BBM, BMM and BBM the protein content was

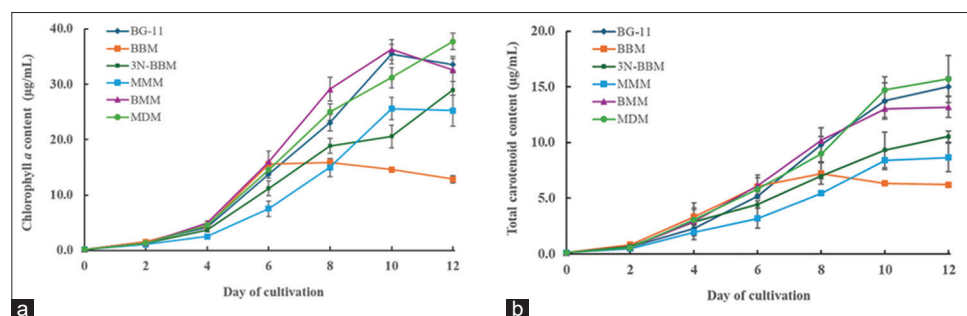
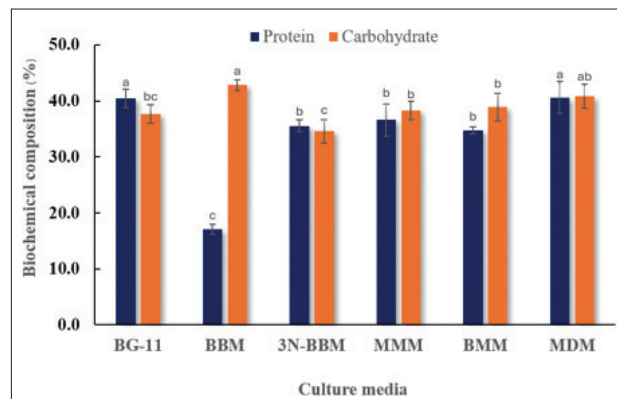


Figure 3: Effect of culture media on the photosynthetic pigments of *T. dimorphus* NUAC202 a) Chlorophyll *a* content and b) Total carotenoid content

Table 1: Total carotenoid content, chlorophyll *a*, lutein, β -carotene and biomass of *T. dimorphus* NUAC202 grown in different media at 12th day of cultivation

Media	Total carotenoids ($\mu\text{g/mL}$)	Chlorophyll <i>a</i> ($\mu\text{g/mL}$)	Lutein (mg/g DW)	β -carotene (mg/g DW)	Biomass (g/L)
BG-11	15.02 $\pm$ 0.87 ^{ab}	33.53 $\pm$ 4.28 ^{ab}	2.68 $\pm$ 0.04 ^a	0.76 $\pm$ 0.01 ^a	2.11 $\pm$ 0.10 ^b
BBM	6.21 $\pm$ 0.21 ^d	12.84 $\pm$ 0.70 ^d	1.21 $\pm$ 0.02 ^c	0.18 $\pm$ 0.00 ^c	1.98 $\pm$ 0.03 ^b
3N-BBM	10.54 $\pm$ 0.49 ^c	28.97 $\pm$ 3.52 ^{bc}	2.10 $\pm$ 0.06 ^c	0.55 $\pm$ 0.02 ^c	2.01 $\pm$ 0.14 ^b
MMM	8.66 $\pm$ 1.27 ^c	25.24 $\pm$ 2.78 ^c	1.83 $\pm$ 0.05 ^d	0.39 $\pm$ 0.01 ^d	1.69 $\pm$ 0.13 ^c
BMM	13.19 $\pm$ 0.95 ^b	32.58 $\pm$ 2.03 ^{ab}	2.61 $\pm$ 0.02 ^a	0.60 $\pm$ 0.01 ^b	2.39 $\pm$ 0.27 ^a
MDM	15.72 $\pm$ 2.11 ^a	37.72 $\pm$ 1.50 ^a	2.34 $\pm$ 0.04 ^b	0.56 $\pm$ 0.01 ^c	2.45 $\pm$ 0.06 ^a

Means in the same column followed by the different alphabetical letters are significantly different as determined by Duncan's New Multiple Range Test (DMRT) ($\alpha=0.05$, $n=3$)

**Figure 4:** Effect of culture media on the protein and carbohydrate contents of *T. dimorphus* NUAC202

differed from the obtained biomass. The biomass obtained in MDM, BG-11, MMM, 3N-BBM, BMM and BBM showed 40.64, 40.49, 36.6, 35.6, 34.7 and 17.0% protein content, respectively. On the contrary, carbohydrate content showed the highest value 40.88% in biomass obtained using BBM. It is shown that the production higher than carbohydrate content found in biomass produced by other media, which may result from stress caused by reduced nitrate content in the medium (BBM has 0.25 g/L NaNO_3). Our study is in accordance with Ram *et al.* (2019), wherein low nitrogen content decreased protein accumulation in *Scenedesmus* sp. CCNM 1028 grown in BBM medium. The lowest protein content may be attributed to reduced nitrate in the medium, which disrupts carbon flux essential for the biosynthesis of high-energy compounds such as proteins and lipids. The biochemical parameters of *T. dimorphus* NUAC202 in different culture media are shown in Figure 4.

Conclusions

In our study, cultivating *T. dimorphus* NUAC202 cells using different media showed marked influences on growth, pigment profile and biochemical characteristics. The highest biomass production (2.45 g/L) was achieved in MDM medium. Lutein content (2.68 mg/g DW) and β -carotene (0.76 mg/g DW) were greatly improved in BG11-biomass. Moreover, MDM and BG11-biomass showed higher protein production (40.64 and 40.49%), whereas BBM-biomass showed higher total carbohydrate production (42.82%). It is proposed that MDM and BG-11 media are the optimum medium for *T. dimorphus* NUAC202 cultivation, indicating that these two media are effective for the co-production of lutein and protein.

Author contributions

Piyawat Pongpadung: Conceptualization, Investigation, Validation, Visualization, Funding acquisition, Supervision, Writing – review & editing. Nittaya Saninsi: Formal analysis, Writing – original draft. Sunanthakan Khumman: Methodology, Data curation, Formal analysis. Somrak Rodjaroen: Formal analysis, Validation. Wittaya Tawong: Formal analysis, Validation. Supat Ponza: Formal analysis, Validation. Mahattanee Phinyo: Formal analysis, Validation. Ramaraj Sathasivam: Conceptualization, Investigation, Validation, Visualization, Writing – review & editing. All authors approved the final manuscript.

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References

- Asker, D., Awad, T. S., Beppu, T., & Ueda, K. (2018). Rapid and selective screening method for isolation and identification of carotenoid-producing bacteria. In C. Barreiro & J.-L. Barredo (Eds.), *Microbial Carotenoids* (pp. 143-170). Springer. <https://doi.org/10.1007/978-1-4939-8742-9>
- Bermejo, E., Ruiz-Domínguez, M. C., Cuasmas, M., Vaquero, I., Ramos-Merchante, A., Vega, J. M., Vilchez, C., & Garbayo, I. (2018). Production of lutein, and polyunsaturated fatty acids by the acidophilic eukaryotic microalga *Coccomyxa onubensis* under abiotic stress by salt or ultraviolet light. *Journal of Bioscience and Bioengineering*, 125(6), 669-675. <https://doi.org/10.1016/j.jbiosc.2017.12.025>
- Cerón, M. C., Campos, I., Sanchez, J. F., Ación, F. G., Molina, E., & Fernandez-Sevilla, J. M. (2008). Recovery of lutein from microalgae biomass: development of a process for *Scenedesmus almeriensis* biomass. *Journal of Agricultural and Food Chemistry*, 56(24), 11761-11766. <https://doi.org/10.1021/jf8025875>
- Chang, Y.-R., Lee, D.-J., Chang, J.-S., & Mujumdar, A.-S. (2015). Enhancement of lutein yield from coagulated *Chlorella* sp. ESP-6 with sodium hypochlorite. *Drying Technology*, 33(4), 429-433. <https://doi.org/10.1080/07373937.2014.970261>
- Chokshi, K., Pancha, I., Maurya, R., Paliwal, C., Ghosh, T., Ghosh, A., & Mishra, S. (2016). Growth medium standardization and thermotolerance study of the freshwater microalga *Acutodesmus dimorphus*—a potential strain for biofuel production. *Journal of Applied Phycology*, 28, 2687-2696. <https://doi.org/10.1007/s10811-016-0826-3>
- Dixit, R., Singh, S., Enamala, M. K., & Patel, A. (2022). Effect

- of various growth medium on the physiology and de novo lipogenesis of a freshwater microalga *Scenedesmus rotundus*-MG910488 under autotrophic condition. *Clean Technologies*, 4(3), 733-751. <https://doi.org/10.3390/cleantechnol4030045>
- Dufossé, L. (2018). Microbial pigments from bacteria, yeasts, fungi, and microalgae for the food and feed industries. In A. M. Grumezescu & A. M. Holban (Eds.), *Natural and Artificial Flavoring Agents and Food Dyes* (pp. 113-132). Elsevier. <https://doi.org/10.1016/B978-0-12-811518-3.00004-1>
- George, B., Pancha, I., Desai, C., Chokshi, K., Paliwal, C., Ghosh, T., & Mishra, S. (2014) Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus*—a potential strain for bio-fuel production. *Bioresource Technology*, 171, 367-374. <https://doi.org/10.1016/j.biortech.2014.08.086>
- Gong, M., & Bassi, A. (2016). Carotenoids from microalgae: a review of recent developments. *Biotechnology Advances*, 34(8), 1396-1412. <https://doi.org/10.1016/j.biotechadv.2016.10.005>
- Hong, S.-J., & Lee, C.-G., 2008. Statistical optimization of culture media for production of phycobiliprotein by *Synechocystis* sp. PCC 6701. *Biotechnology and Bioengineering*, 13, 491-498. <https://doi.org/10.1007/s12257-008-0154-9>
- Kochart, A. G. (1978). Carbohydrate determination by the phenol-sulphuric acid method. In J. A. Hellebust & J. S. Craigie (Eds.), *Handbook of Phycological and Biochemical Methods* (pp. 95-97). Cambridge University Press.
- Lee, N., Ko, S.-R., Ahn, C.-Y., & Oh, H.-M. (2018). Optimized co-production of lipids and carotenoids from *Ettlia* sp. by regulating stress conditions. *Bioresource Technology*, 258, 234-239. <https://doi.org/10.1016/j.biortech.2018.03.006>
- Lin, J.-H., Lee, D.-J., & Chang, J.-S. (2015). Lutein production from biomass: marigold flowers versus microalgae. *Bioresource Technology*, 184, 421-428. <https://doi.org/10.1016/j.biortech.2014.09.099>
- Liu, Y. (2018). Optimization study of biomass and astaxanthin production by *Haematococcus pluvialis* under minkery wastewater cultures.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275. [https://doi.org/10.1016/S0021-9258\(19\)52451-6](https://doi.org/10.1016/S0021-9258(19)52451-6)
- Mandalam, R. K., & Palsson, B. (1998). Elemental balancing of biomass and medium composition enhances growth capacity in high-density *Chlorella vulgaris* cultures. *Biotechnology Bioengineering*, 59(5), 605-611. [https://doi.org/10.1002/\(SICI\)1097-0290\(19980905\)59:5<605::AID-BIT11>3.0.CO;2-8](https://doi.org/10.1002/(SICI)1097-0290(19980905)59:5<605::AID-BIT11>3.0.CO;2-8)
- Ördög, V., Stirk, W. A., Bálint, P., van Staden, J., & Lovász, C. (2012). Changes in lipid, protein and pigment concentrations in nitrogen-stressed *Chlorella minutissima* cultures. *Journal of Applied Phycology*, 24(4), 907-914. <https://doi.org/10.1007/s10811-011-9711-2>
- Piccaglia, R., Marotti, M., & Grandi, S. (1998). Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. *Industrial Crops and Products*, 8(1), 45-51. [https://doi.org/10.1016/S0926-6690\(97\)10005-X](https://doi.org/10.1016/S0926-6690(97)10005-X)
- Procházková, G., Brányiková, I., Zachleder, V., & Brányik, T. (2014). Effect of nutrient supply status on biomass composition of eukaryotic green microalgae. *Journal of Applied Phycology*, 26, 1359-1377. <https://doi.org/10.1007/s10811-013-0154-9>
- Ram, S., Paliwal, C., & Mishra, S. (2019). Growth medium and nitrogen stress sparked biochemical and carotenogenic alterations in *Scenedesmus* sp. CCNM 1028. *Bioresource Technology Reports*, 7, 100194. <https://doi.org/10.1016/j.biteb.2019.100194>
- R Core Team (2025). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Sanpapao, P., Tawong, W., Pongpadung, P., Ponza, P., & Ponza, S. (2023). Effect of phosphorus and sodium chloride levels on growth performance, carotenoid accumulation and isomerization to 9-cis β -carotene in Thai *Dunaliella salina* NUAC09. *International Aquatic Research*, 15, 27-38.
- Sathasivam, R., & Ki, J.-S. (2018). A review of the biological activities of microalgal carotenoids and their potential use in healthcare and cosmetic industries. *Marine Drugs*, 16(1), 26. <https://doi.org/10.3390/md16010026>
- Sathasivam, R., Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2019). Microalgae metabolites: A rich source for food and medicine, 26(4), 709-722. <https://doi.org/10.1016/j.sjbs.2017.11.003>
- Sathasivam, R., Radhakrishnan, R., Kim, J. K., Park, S. U. (2021). An update on biosynthesis and regulation of carotenoids in plants. *South African Journal of Botany*, 140, 290-302. <https://doi.org/10.1016/j.sajb.2020.05.015>
- Sterner, R. W., & Grover, J. P. (1998). Algal growth in warm temperate reservoirs: kinetic examination of nitrogen, temperature, light, and other nutrients. *Water Research*, 32(12), 3539-3548. [https://doi.org/10.1016/S0043-1354\(98\)00165-1](https://doi.org/10.1016/S0043-1354(98)00165-1)
- Xie, Y., Ho, S.-H., Chen, C.-N. N., Chen, C.-Y., Ng, I.-S., Jing, K. J., Chang, J.-S., & Lu, Y. (2013). Phototrophic cultivation of a thermo-tolerant *Desmodesmus* sp. for lutein production: effects of nitrate concentration, light intensity and fed-batch operation. *Bioresource Technology*, 144, 435-444. <https://doi.org/10.1016/j.biortech.2013.06.064>