Effects of Different Light Intensities on Growth and Fucoxanthin Concentration of *Fistulifera solaris*

Zahra Vafaei Naeini[1], Mindy McCallum[1#] and Ruth Ndathe[1#]

[1]Louisiana State University
[#]Advisor

ABSTRACT

Biofuel production using algae is believed to have tremendous positive impacts on the environment, and it seems a promising solution for global warming. However, growing algae on a large scale requires many resources making mass production of biofuels not yet economically viable. In this study, an oleaginous diatom, *Fistulifera solaris,* was cultured in autotrophic conditions under four light intensities for 14 days to determine which conditions allowed for higher cellular growth and higher fucoxanthin concentration, a valuable photosynthetic pigment that can make the process of biofuel production affordable. As a measure of abundance, absorbance values of diatoms in 750 nm wavelength (OD 750) were obtained on days 0, 7, and 14. On day 14, fucoxanthin concentration was measured. Statistical analyses revealed a significant change in the growth of diatoms under the four treatments that may be explained by varying rates of reproduction of the diatoms grown under different light intensities. Specifically, the highest OD 750 and growth were observed in medium-high light intensity, while the highest fucoxanthin concentration was observed in low light intensity. It is suggested that diatoms grown in lower light intensities may be ideal for increased fucoxanthin concentration because the geranyl-geranyl diphosphate synthase (*GGPPS*) gene, which is responsible for encoding a fucoxanthin precursor, is downregulated in exposure to high light intensities. Considering this data, low light intensity leads to the highest fucoxanthin concentration, and medium-high light intensity leads to the highest OD 750 and maximum growth under autotrophic conditions.

Introduction

Algae can be beneficial to humans in various ways; in human or animal nutrition, in the pharmaceutical and health industry, in bioplastic production, and more importantly, they can be utilized as a great source of biofuel. All these products are environmentally friendly (Arica et al., 2017). Algae can also trap CO_2 that is produced in many industries, and therefore, producing algae on a large scale can be helpful in fighting global warming (Sirisuk et al., 2018). However, mass production of algae requires a lot of energy and resources, resulting in its production on a mass scale not yet economically viable (Hannon et al., 2010).

Mass production of algae can become economically viable, however, by extracting valuable products from them and using these products to offset the high cost of production. An example of such a product is fucoxanthin, which is a photosynthetic pigment found in marine organisms, such as brown algae and microalgae. Fucoxanthin exists in the thylakoid membrane and transfers light energy to the electron transport chain of photosynthesis through chlorophyll a (Gómez-Loredo et al., 2015). Fucoxanthin has broad usage and is costly in pharmaceutical applications, including anti-cancer, anti-obesity, and antidiabetic activity (Nur et al., 2018). Therefore, to make the large-scale production of algae more economical, the algae should be grown in conditions that yield the highest growth rate, and the maximum fucoxanthin concentration.

One diatom species that seems a promising candidate for producing bioenergy is the oleaginous diatom *Fistulifera* sp. strain JPCC DA0580 or *Fistulifera solaris*, a diatom native to Japan (Sato et al., 2014). Microalgae such as diatoms have simpler growth requirements compared to other algae, such as brown algae. Microalgae have simpler structures, and as a result, they consume their energy directly for growth, reproduction processes, and photosynthesis and not for maintaining complex structures. Microalgae, compared to terrestrial plants, grow 100 times faster. They can also use CO_2 much more efficiently than plants and, thus, fix CO_2, transforming solar energy into chemical energy (Gómez-Loredo et al., 2015). Thus, among photosynthetic organisms that have fucoxanthin, microalgae are a better option for biofuel production. Diatoms are a group of microalgae; they are autotrophic microorganisms and to grow they need nutrients (e.g., phosphorus, and nitrogen), light, and CO_2 for photosynthesis (Sirisuk et al., 2018). Diatoms are promising candidates for bioenergy production because they can produce sustainable energy without massive CO_2 release. In addition, producing biofuel using diatoms is possible throughout the year continuously, while it is not possible when using green algae (Matsumoto et al., 2017).

This diatom species has advantageous characteristics for producing biofuels, such as a high growth rate which sets it apart from other similar species. Among microalgae, some species require at least 2 weeks to get to the plateau of growth, while *Fistulifera solaris* requires only 1 week. Another beneficial characteristic of this strain is high neutral lipid content (40-60%, w/w). Like *Chlorella* sp. or *Nannochloropsis* sp., this strain has a high yield of oil production. Considering triacylglycerol, this strain primarily makes low unsaturated-degree fatty acids methyl esters, such as methyl palmitoleate (C16:1) and methyl palmitate (C16:0), and can make quality biofuel (Sato et al., 2014). In addition to demonstrating high lipid content, this strain is also able to grow in various temperatures. This is important because the energy balance of biofuel production can dissipate because of controlling the temperature of a massive culture media for large-scale production of microalgae since it requires a huge amount of energy. From an economic perspective and to reduce energy consumption, it is preferred not to manipulate the temperature of the culture. Evidently, a previous study performed through outdoor culturing in Japan without controlling the temperature of the culture showed that this strain exhibited stable growth in different types of bioreactors from April to November (Matsumoto et al., 2017). Another factor that should be considered when culturing this strain for bioenergy purposes is the effects of different light intensities on the fucoxanthin concentration of this diatom.

Accumulation of fucoxanthin and growth in diatoms depend on environmental factors and cultural conditions (H. Wang et al., 2018). It is crucial to evaluate the impacts of the intensity of light on the accumulation of pigment when we use pigments as valuable products to fund large-scale biofuel production (Nur et al., 2018). Light provides the energy for all biochemical processes, particularly photosynthesis. Thus, it is believed that as light intensity increases, phototropic organisms grow more rapidly. However, when present in high intensities, light can damage cells (Gómez-Loredo et al., 2015). A previous study demonstrated that when exposed to light for a long time, the growth rate of *Nannochloropsis* sp. was reduced because of the photoinhibition effect (Wahidin et al., 2013). In another study, it was shown that in autotrophic conditions, the cell growth of the marine diatom *Phaeodactylum tricornutum* decreased in high light intensity, which could be due to photoinhibition (Yang & Wei, 2020). When a particular light intensity inhibits photosynthesis, increasing light intensity would not positively impact the growth any further. Thus, the growth of cells is reduced in response to high light intensity (H. Wang et al., 2018). Also, since fucoxanthin is a pigment that harvests light, fucoxanthin content can be impacted by light intensity (Nur et al., 2018). Yang & Wei (2020) demonstrated that fucoxanthin accumulation is favored in low light intensity. They demonstrated that under autotrophic conditions in *P. tricornutum*, with the increase of light intensity, fucoxanthin contents dropped significantly. Also, geranyl geranyl diphosphate (GGPP) is a precursor for fucoxanthin production. The transcriptome analysis has shown that enzymes involved in the biosynthesis of this precursor are encoded by genes that are downregulated under high light intensity, and this leads to a decrease in fucoxanthin concentration (Yang & Wei, 2020). Thus, with a range of light intensities, increasing light intensity promotes growth to a certain point but decreases the accumulation of fucoxanthin (H. Wang et al., 2018).

However, the literature on the effects of different light intensities on fucoxanthin concentration and the growth of this diatom strain is scarce. This study was conducted to evaluate the impact of four different light intensities on the growth and fucoxanthin concentration of *Fistulifera solaris*. The diatoms in this study are cultured in F/2 media that contains all the essential nutrients; they can grow and reproduce via autotrophic metabolism. It is predicted that lower light intensities will lead to higher fucoxanthin concentrations of *Fistulifera solaris* in autotrophic conditions.

Also, since diatoms are cultured in a media containing all the essential nutrients, they can grow and reproduce, so it is predicted that OD 750 values and, thus, the growth of *Fistulifera solaris* will increase in each light intensity. In addition, it is expected that OD 750 values increase as light intensity increases. It is predicted that the growth might reach a plateau in high light intensity if photoinhibition occurs.

Methods

Experimental Design

To study the effect of light intensities on growth and fucoxanthin concentration, cultures of *Fistulifera solaris* were grown in F/2 media under four different light intensities: Low (L; 74 LUX), Medium Low (ML; 4300 LUX), Medium High (MH; 6400 LUX), and High (H; 10,000 LUX). Three cultures of 1 mL of diatoms and 3 mL of F/2 media were incubated for 14 days beneath each light intensity, and absorbance values were measured at 750 nm wavelength on day 0, day 7, and day 14. Diatoms cannot absorb light at 750 nm wavelength, so it is a proper wavelength to estimate the concentration of diatoms and check their growth.

Fucoxanthin Concentration of the Diatoms

At the conclusion of the experiment on day 14, the weight of cells and fucoxanthin concentrations were measured. OD 750 from day 14 only determined the number of cells and not the change in cell sizes. The weight of cells, however, also accounts for changes in cell size. To obtain the weight of cells, samples were filtered through grade 42 Whatman filter papers (2.3 μm) using the Filtr8 vacuum filtration kit. To remove the excess media and dry the filter papers, they were then placed in an incubator for 5 minutes. Then the weights of the filter papers (weight of filter paper and diatoms) were measured again. Subtracting these two numbers resulted in the weight of diatoms.

To obtain fucoxanthin concentration, the pigment was extracted in ethanol, and absorbance was measured. The filter papers were cut into small pieces and placed into separate test tubes, one for each light intensity. To lyse the diatom cells and dissolve the pigments inside them, 4 mL of 100 % ethanol was added to each test tube, and the solution was vortexed until the filter paper turned white. The extract was slowly transferred into clean test tubes covered with foil. Using a Spectronic 200, absorbance values at 445 nm and 663 nm were taken quickly (to prevent pigments from disintegration from light). Absorbance at 445 nm indicates absorbance of both chlorophyll and fucoxanthin, while absorbance values taken at 663 nm indicate that of chlorophyll alone. Thus, subtracting the two allows for the absorbance of fucoxanthin to be determined. To calculate the concentration of fucoxanthin, an equation from L. Wang et al., 2018 was used (Equation 1).

Equation 1: Calculation of fucoxanthin concentration (CFx):

$$\mathrm{CFx}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) = \frac{\big((6.39 * \text{ Absorbance at 445nm}) - (5.18 * \text{ Absorbance at 663nm})\big)}{\mathrm{OD\ 750}}$$

Data Analysis

To analyze the effect of different light intensities on the growth (OD 750) and fucoxanthin concentration of diatoms grown in F/2 media, analyses were conducted in Excel. To visualize how OD 750 values changed over time for each treatment, a line graph of means and standard errors was calculated. The significance of the slope was also determined. The p-values of each slope for OD 750 over time were calculated. Then, to compare the growth of diatoms in different treatments with each other, p-values of OD 750 mean values for readings on days 0, 7, and 14 were obtained through analysis of variance (ANOVA). p-value ≤ 0.05 deduced a significant difference.

Results

Rate of Growth Determined by OD 750 Values

In all four different light intensities, OD 750 of *Fistulifera solaris* significantly increased from day 0 to day 14 (Table 1), and this shows that all the different light intensities were favorable for diatom growth. However, the medium-high light intensity yielded the highest growth (Table 1) and showed the fastest rate of growth, as shown by the slope of the trendlines in (Table 2) and in (Figure 1). This was followed closely by the low light intensity, and then the medium-low and high light intensities were third and fourth, respectively, in the rate of growth (Table 2) and (Figure 1). This suggests that the best light intensities that would yield high and fast growth rates were medium-high and low light intensities.

Table 1. Means of growth (OD 750) values of *Fistulifera solaris* in four treatments of light intensity on days 0, 7, and 14. There were a total of three replicates per treatment.

samples	Day 0	Day 7	Day 14
	Mean	Mean	Mean
High	0.180000	0.363333	0.523333
M High	0.200000	0.500000	0.676667
M Low	0.203333	0.376667	0.600000
Low	0.186667	0.460000	0.663333

Table 2. Slopes of the trendlines of *Fistulifera solaris* growth (OD 750). Diatoms were grown in F/2 media under four treatments of light intensity (3 replicates each). p-values of all the slopes are <0.0001 ($\alpha = 0.05$).

Samples	Slopes of linear trendlines
High	0.1717
Medium High	0.2383
Medium Low	0.1983
Low	0.2383

Fucoxanthin Concentration

For fucoxanthin concentration, the lower light intensities had higher fucoxanthin concentrations than the higher light intensities. Specifically, the lower light intensity had the highest fucoxanthin concentration at 11.16 mg/L, while the medium-high had the lowest fucoxanthin concentration at 3.36 mg/L, which was less than half that obtained in the low light intensity (Figure 2). This suggests that lower light intensities favor high fucoxanthin production. However, further analysis on this should be conducted since the data shown is only from one sample replicate.

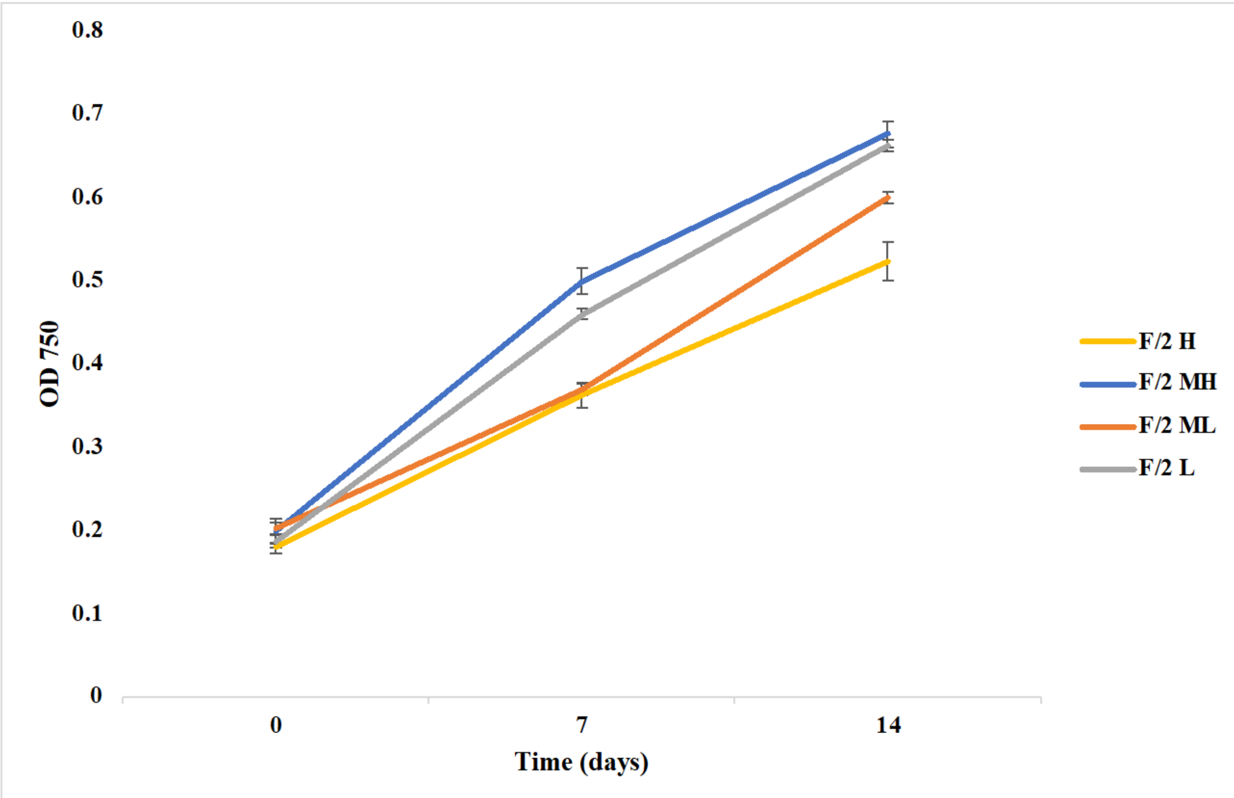

Figure 1. Growth (OD 750) of *Fistulifera solaris* under different light intensities. Values include means and standard errors of diatoms in four treatments of light intensity, including low, medium-low, medium-high, and high light intensity, at readings on days 0, 7, and 14. There were a total of three replicates per treatment. Absorbance values were taken with a Spectronic 200 at 750 nm.

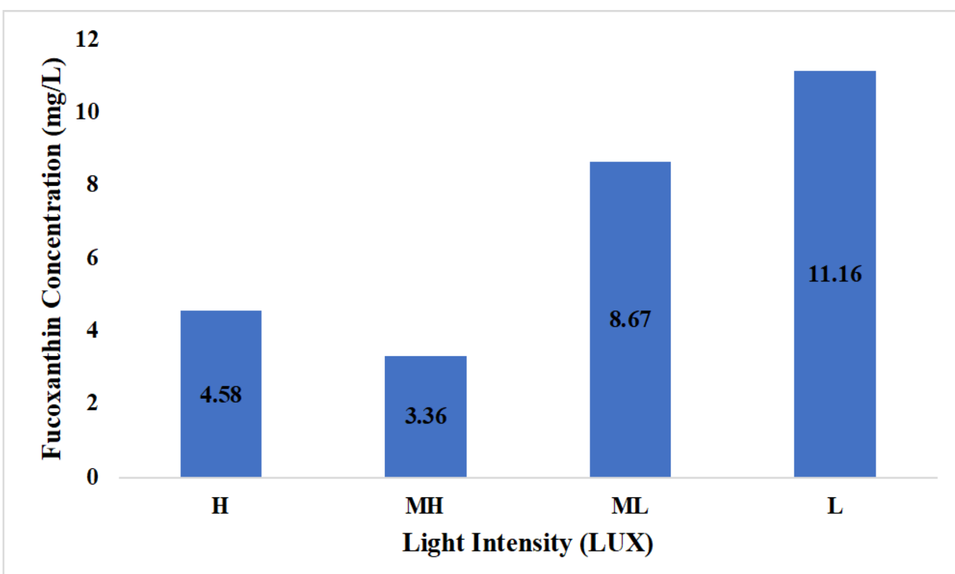

Figure 2. Fucoxanthin concentration (in mg/L) of diatoms in four different treatments each with F/2 media and four different light intensities, including low, medium-low, medium-high, and high light intensity. There was a total of three replicates per treatment and the fucoxanthin concentration of each three replicates was measured together on day 14 (data shown, therefore is n=1)

Discussion

In this study, *Fistulifera solaris* was cultured in F/2 media and four different light intensities with the goal of finding a proper light intensity to achieve the highest growth and fucoxanthin concentration to make the mass production of biofuels affordable. Based on the dependency of diatom growth on photosynthesis and therefore light intensity in the autotrophic condition it was hypothesized that an increase in light intensity should have a positive influence on growth. Also, because the genes encoding enzymes in the biosynthesis of geranyl-geranyl diphosphate (GGPP), which is the precursor of fucoxanthin, are downregulated in exposure to high light intensity in *Phaeodactylum tricornutum* (Yang & Wei, 2020) it was expected to observe the same process in *Fistulifera solaris* and a decrease in fucoxanthin concentration in response to increase in light intensity was predicted.

The expected significant difference in the growth of diatoms among all four treatments is explained by varying rates of reproduction of diatoms under different light intensities. In alignment with our prediction, OD 750 values increased as light intensity increased; however, in high light intensity, there was a sharp decrease in OD 750 value, likely due to the photoinhibition effect (Yang & Wei, 2020). Similarly, in a previous study, a decrease in the cell growth of diatoms in high light intensity under autotrophic conditions was observed (Yang & Wei, 2020). Medium-high light intensities, on the other hand, showed the highest growth, and indicator average light intensities would be best for the growth of the diatoms. On the other hand, the highest fucoxanthin concentration was observed in low light intensity. This could be explained by considering the activation of non-photochemical quenching (NPQ) in exposure to high light intensity. The diadinoxanthin cycle in diatoms is an important part of NPQ, which protects cells from damage when exposed to high light intensity by transforming extra light energy into heat energy. High light intensity stimulates the conversion of diadinoxanthin to diatoxanthin and violaxanthin to zeaxanthin, which ultimately leads to the reduction of fucoxanthin concentration (Yang & Wei, 2020).

Conclusion

As the data suggests, it is hard to find a proper light intensity that yields both high growth and high fucoxanthin concentration while culturing *Fistulifera solaris* in autotrophic media. However, the low light intensity would be the best to use for further studies since it showed the highest fucoxanthin concentration and the second best in growth. Using low light intensities in large-scale bioreactors would reduce energy costs, further reducing the overall cost of algae large-scale production. Previous studies have shown that diatoms grown in mixotrophic conditions, in which diatoms have both autotrophic and heterotrophic metabolism, at low light intensities show enhanced growth in comparison to photoautotrophic conditions (Garcia et al., 2005). Further studies could determine the concentration of fucoxanthin under these conditions. In conclusion, this study narrowed down the possible options for the mass production of biofuels using *Fistulifera solaris*, which facilitates future studies on the best condition to grow this microalga for biofuels.

Limitations

One factor that might have influenced the results could be the inability to provide aeration, which is a very important aspect for the growth of any microorganism, including diatoms, through continuous shaking or stirring. Aeration has also been shown to have positive impacts on fucoxanthin concentration (Gómez-Loredo et al., 2015). Thus, lack of aeration could have resulted in lower OD 750 values and fucoxanthin concentrations.

Acknowledgments

We thank the Kato Lab, the Introductory Biology Program in the Department of Biological Sciences, as well as the LSU College of Sciences for supporting this research.

References

Arica, Ş. Ç., Demirci, S., & Ozyilmaz, A. (2017). A study on the rich compounds and potential benefits. *The Pharma Innovation Journal*. Retrieved November 25, 2022, from https://www.thepharmajournal.com/archives/2017/vol6issue12/PartA/6-12-85-256.pdf

Cerón Garcıá M.C., Sánchez Mirón, A., Fernández Sevilla, J. M., Molina Grima, E., & Garcıá Camacho F. (2005). Mixotrophic growth of the microalga *Phaeodactylum tricornutum*. *Process Biochemistry*, *40*(1), 297–305. https://doi.org/10.1016/j.procbio.2004.01.016

Gómez-Loredo, A., Benavides, J., & Rito-Palomares, M. (2015). Growth Kinetics and fucoxanthin production of *Phaeodactylum tricornutum* and *Isochrysis galbana* cultures at different light and agitation conditions. *Journal of Applied Phycology*, *28*(2), 849–860. https://doi.org/10.1007/s10811-015-0635-0

Hannon, M., Gimpel, J., Tran, M., Rasala, B., & Mayfield, S. (2010). Biofuels from algae: Challenges and potential. *Biofuels*, *1*(5), 763–784. https://doi.org/10.4155/bfs.10.44

Matsumoto, M., Nojima, D., Nonoyama, T., Ikeda, K., Maeda, Y., Yoshino, T., & Tanaka, T. (2017). Outdoor cultivation of marine diatoms for year-round production of biofuels. *Marine Drugs*, *15*(4), 94. https://doi.org/10.3390/md15040094

Nur, M. M., Muizelaar, W., Boelen, P., & Buma, A. G. (2018). Environmental and nutrient conditions influence fucoxanthin productivity of the marine diatom *Phaeodactylum tricornutum* grown on palm oil mill effluent. *Journal of Applied Phycology*, *31*(1), 111–122. https://doi.org/10.1007/s10811-018-1563-6

Sato, R., Maeda, Y., Yoshino, T., Tanaka, T., & Matsumoto, M. (2014). Seasonal variation of biomass and oil production of the oleaginous diatom *Fistulifera* sp. in outdoor vertical bubble column and Raceway-type bioreactors. *Journal of Bioscience and Bioengineering*, *117*(6), 720–724. https://doi.org/10.1016/j.jbiosc.2013.11.017

Singh, S. P., & Singh, P. (2015). Effect of temperature and light on the growth of algae species: A Review. *Renewable and Sustainable Energy Reviews*, *50*, 431–444. https://doi.org/10.1016/j.rser.2015.05.024

Sirisuk, P., Ra, C.-H., Jeong, G.-T., & Kim, S.-K. (2018). Effects of wavelength mixing ratio and photoperiod on microalgal biomass and lipid production in a two-phase culture system using LED illumination. *Bioresource Technology*, *253*, 175–181. https://doi.org/10.1016/j.biortech.2018.01.020

Wahidin, S., Idris, A., & Shaleh, S. R. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. *Bioresource Technology*, *129*, 7–11. https://doi.org/10.1016/j.biortech.2012.11.032

Wang, H., Zhang, Y., Chen, L., Cheng, W., & Liu, T. (2018). Combined production of Fucoxanthin and EPA from two diatom strains *Phaeodactylum tricornutum* and *Cylindrotheca fusiformis* cultures. *Bioprocess and Biosystems Engineering*, *41*(7), 1061–1071. https://doi.org/10.1007/s00449-018-1935-y

Wang, L.-J., Fan, Y., Parsons, R., Hu, G.-R., Zhang, P.-Y., & Li, F.-L. (2018). A rapid method for the determination of fucoxanthin in diatom. *Marine Drugs*, *16*(1), 33. https://doi.org/10.3390/md16010033

Yang, R., & Wei, D. (2020). Improving fucoxanthin production in mixotrophic culture of marine Diatom *Phaeodactylum tricornutum* by LED light shift and nitrogen supplementation. *Frontiers in Bioengineering and Biotechnology*, *8*. https://doi.org/10.3389/fbioe.2020.00820