









# Evaluation of *Spirulina platensis* Growth Factors for High Efficiency Biomass Production Running Title: Evaluation of *Spirulina Platensis* Growth Factors

Nahid Ghaed Amini <sup>1</sup>, Ali Choopani <sup>1,\*</sup>, Amir Hossein Choopani <sup>2</sup>, Mahdi Ghorbanalizadeghan <sup>3</sup>, Mohammad Fazilati <sup>4</sup>, Hossein Salavati <sup>4</sup>, Ali Mohammad Latifi <sup>5</sup>, Hamid Reza Shiran <sup>6</sup>

<sup>1</sup> Amiran Oghyanoos Biotechnology Company, Tehran, Iran; nahid.amini59@gmail.com (N.G.A); abrcs@bmsu.ac.ir (A.C);

<sup>2</sup> Department of Environmental Science, University of Agricultural Sciences and Natural Resources, Gorgan, Iran; amirch.1382@gmail.com;

<sup>3</sup> Biozimee Company, Qazvin, Iran; alizadehgann@gmail.com;

<sup>4</sup> Department of Biochemistry, Faculty of Biological Science, Payame Noor University, Tehran, Iran; m.fazilati@gmail.com (M.F); hosseinsalavati@yahoo.com (H.S);

<sup>5</sup> Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran; amlatifi290@gmail.com;

<sup>6</sup> Department of Environmental Engineering, School of Environmental, University of Tehran, Iran; hrshiran@gmail.com;

\* Correspondence: abrcs@bmsu.ac.ir;

Received: 13.05.2023; Accepted: 11.08.2023; Published: 25.11.2025

**Abstract:** *Spirulina platensis* is an important microalga that has been cultivated commercially in many tropical countries and sub-tropical and temperate regions to feed humans and animals. The cost of culture medium accounts for 15-20% of the production process. Lower production costs can be a fundamental solution for the high-value production of *Spirulina*. Therefore, it is necessary to optimize. This study investigated the optimization of the growth of *Spirulina* as an Iranian species. Samples were isolated from a specific region (North of Iran). More than 130 culture mediums were studied with different gradients such as temperature, light, PH, and essential salts. The results showed that the best growth conditions were in culture medium No. 18, at 25°C, pH 10.5, and light intensity of 3500 LUX, with a dry mass of 40 mg per 100 ml. This study achieved the best performance and optimized environmental conditions for *Spirulina platensis*.

**Keywords:** *Spirulina platensis*; biomass; culture; optimization.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

## 1. Introduction

In recent years, microalgae have been common in human life [1]. They have been found to be the first microorganisms to produce oxygen on Earth. More than 5.4 million tons of algae (worth 4.9 billion dollars) are cultured and harvested annually for various purposes [2]. Studies show that more than 185 species of algae have been consumed as food, including green algae (25 species), brown algae (70 species), and red algae (90 species) [3]. In addition, researchers have been interested in identifying the features of valuable compounds with medicinal properties produced by photosynthetic organisms [4]. Algae have been considered a powerful

source to produce a variety of vitamins, unsaturated fatty acids, and significant groups of compounds with therapeutic activity [3, 4]. Algae can build organic matter, and they can grow fast. It can be achieved in large volumes of biological mass with limited amounts of valuable compounds at low cost and in a short time [5]. Different types of biomass can be produced for human food, such as *Chlorella*, *Sandemos*, *Donalila*, *Spirulina*, *Nostoc*, *Anafizomnon*, and *Profiridium* [1, 6]. *Spirulina* has produced about 2000 to 3000 tons per year, and the majority of its production is planted in the outdoor pool. Algal production has been used as a source of protein food supplements in many industrial countries [7, 8]. In recent decades, 75 percent of microalgae biomass has been used to produce powders, tablets, capsules, and pastilles [9, 10]. In addition, various algae extracts are produced as the second generation of microalgae products [11]. Nowadays, the importance of microalgae is increasing as a source of high-protein food [10], [12, 13]. *Chlorella*, *Sndesmos*, and *Spirulina* are used as veterinary poultry, fish, pig, and silkworm food [14]. The development of systems has been considered for the cultivation of microalgae on a large scale since 1940 [15, 16]. The main effort is to achieve the appropriate balance among the factors affecting the Lake's growth, such as light, temperature, nutrients, pH, and mixing. The light should be a limiting factor to achieve the maximum biomass [15]. The theoretical maximum conversion efficiency for optical energy is 6.6%, but an acceptable level is lower; for example, *Spirulina* at high production rates converts solar energy at 1% efficiency. Some nutrients are necessary for algae growth in culture media. These materials can include nutrients, trace elements, and vitamins and can vary depending on the target species and typical products. Biomass production increases with the addition of an organic carbon source, and the type of carbon source used depends on pH [2]. The ideal temperature should be an appropriate temperature for the growth of the next (usually between 15-30°C) [17, 18]. Several different models are important in the growth parameters. Also, there are systems computer technology for viewing and controlling factors such as carbon dioxide and nutrients [19]. Although, it is possible that the conditions should be achieved for maximum biomass, this needs may be different according to the final product such as lipids, pigments and especially when the product is a result of the second metabolism [20, 21]. This study investigated the optimization growth of *Spirulina platensis* as an Iranian species. Iran microalgae can build organic matter and they can grow fast in this area. In recent years, their cultivation has increased.

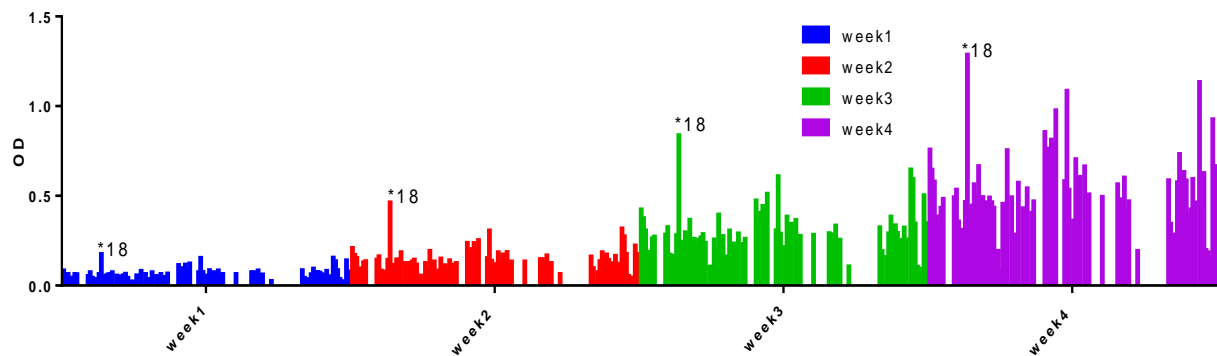
## 2. Materials and Methods

Strains were isolated from a pond in the North of Iran (WGS84: 37° 20' 59" N, 54° 35' 2" E) and then identified at the Center of Shahid Beheshti University using morphological, physiological, and biological laboratory keys. And cultivated with different volumes of Parameters. 130 different cultures' media used for culturing. In addition, media cultures were randomly selected from nine materials: NaHCO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, NaNO<sub>3</sub>, K<sub>2</sub>SO<sub>4</sub>, NaCl, MgSO<sub>4</sub>, CaCl<sub>2</sub>, FeSO<sub>4</sub>, and EDTA. A UV lamp was used to sterilize the culture room for about half an hour. Arlene was washed with distilled water and sterilized in Avon at 100°C to dry for one hour. For mass culture, 10ml of pure algae containing  $9 \times 10^8$  (cells /mL) were inoculated onto Arlene 250ml containing sterile culture media and maintained with different environmental conditions for 30 days. Four areas of material concentration, pH gradient, Tm, and light were studied. OD, cell counts, and dry weight were evaluated daily. In the first stage, the gradient of salt concentration was investigated. 130 different concentrations of salt were prepared with different concentrations of salt in 250ml (Tm: 25±0/5°C, pH: 9, light intensity: 4000 LUX,

lightness /darkness: 16/8). Finally, the most suitable cultivation environment (cell counts and OD) was selected (culture number: 18). Then, a pH gradient of 7-8 and 12 was tested for a month, and OD, counts, and dry weight were determined for a month [22, 23]. The suitable temperature was 20 to 40 degrees for different growths fixed in the box. OD in 560nm, cell counts, and dry weight were measured with the mentioned method. In this study, the main variables were light intensity (quantity of light), lighting time, and darkness. The impact of three light intensities was investigated in 2000 to 6000 Lux during the period of light (16/8).

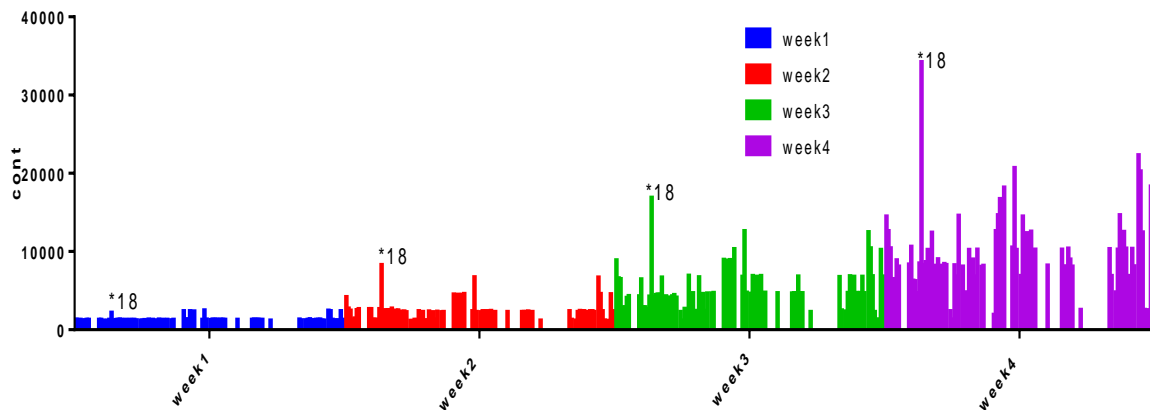
### 3. Results and Discussion

Culture 18 had the highest absorption among 130 cultures in the first, second, third, and fourth weeks, as shown in Figure 1.



**Figure 1.** Absorption OD of culture number 18.

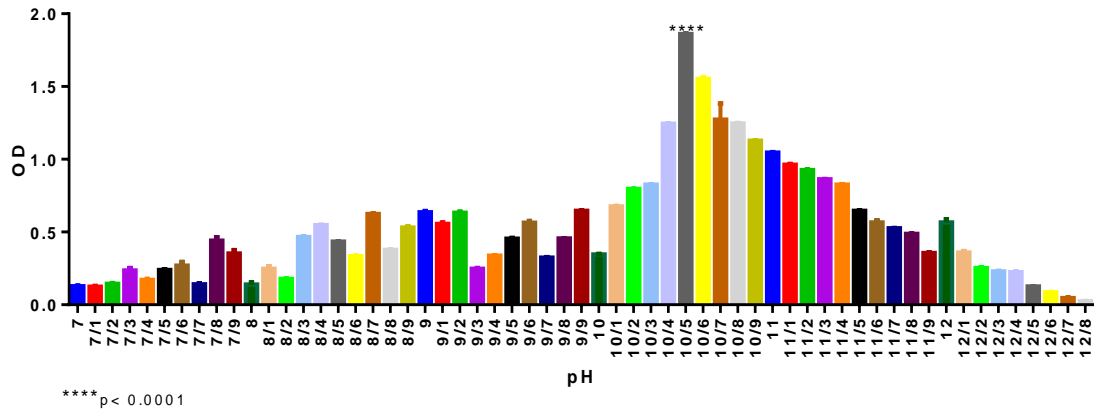
Growth optimization was not detected in the first week, but it gradually increased, reaching its highest level in the fourth week. The results of the growth optimization of culture number 18 are shown in Figure 2.



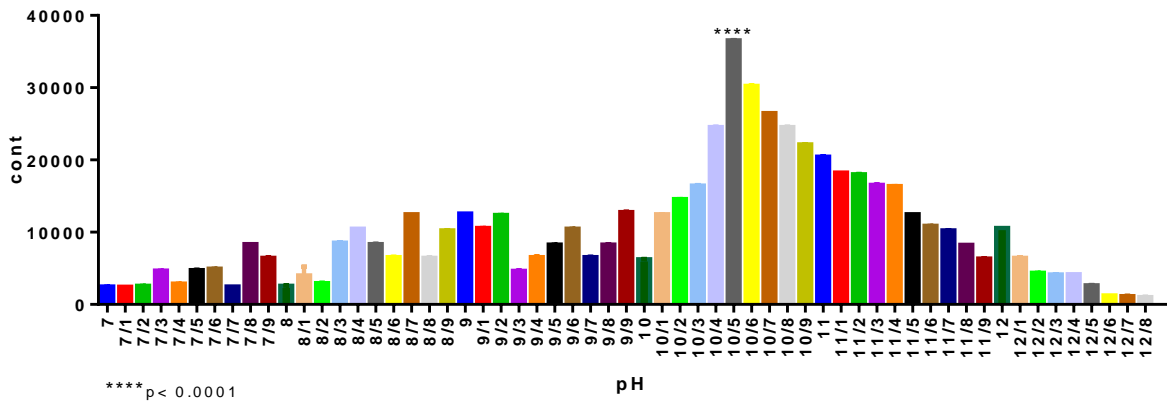
**Figure 2.** Cell counts of Culture number 18.

Figure 3 shows that the best pH for growth optimization was 10.5 (over 40 mg/100 ml). The results of the growth optimization of algae with pH factor, cell counting, and suffering (7-8.12), and the preparation of dry weight are shown in Figures 4 and 5.

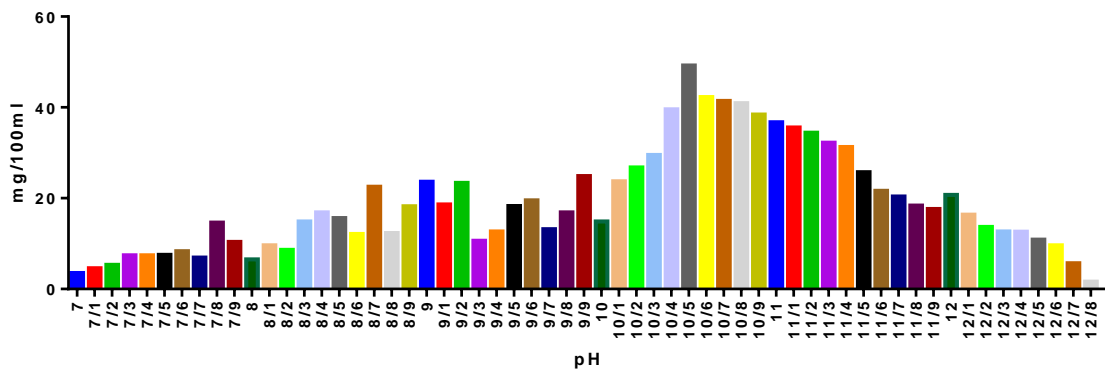
The results of the growth optimization of algae with Tm factor, cell counting, and dry weight are shown in Figures 6, 7, and 8.



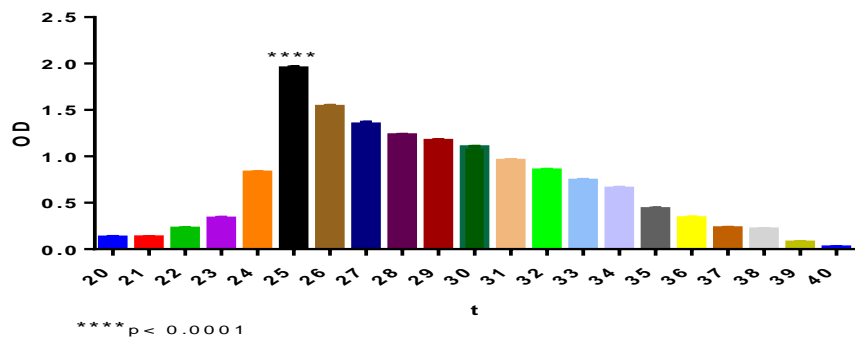
**Figure 3.** The algae grow with the pH factor.



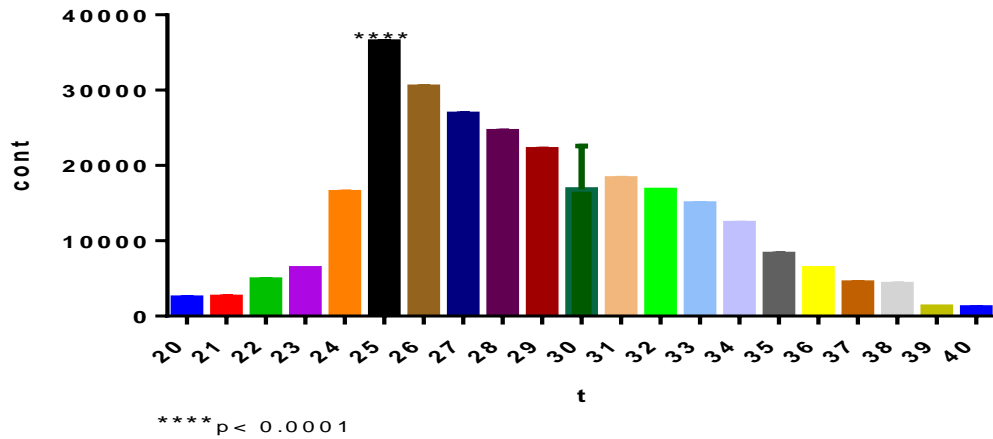
**Figure 4.** The algae growth with the pH factor and cell counting.



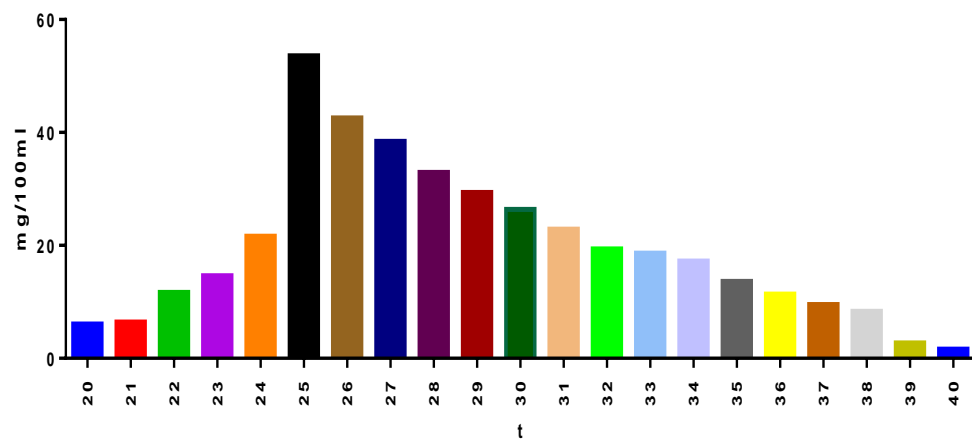
**Figure 5.** The algae growth with the pH factor in suffering (7-8.12) and the preparation of dry weight



**Figure 6.** The growth optimization of algae with the Tm factor.

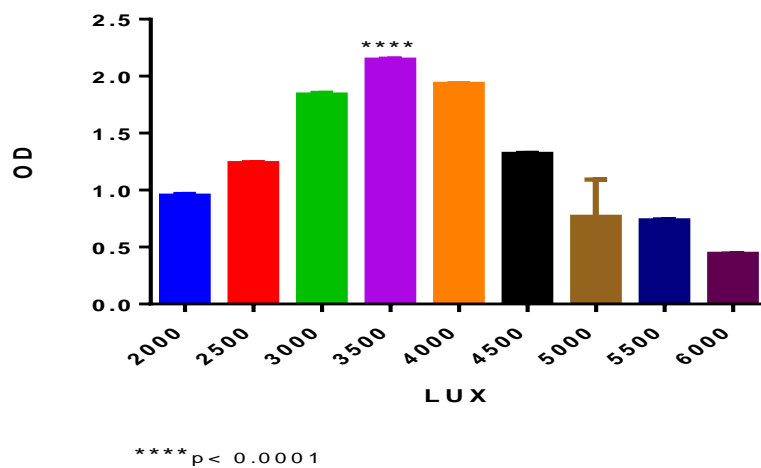


**Figure 7.** The growth optimization of algae with the Tm factor and cell counting.

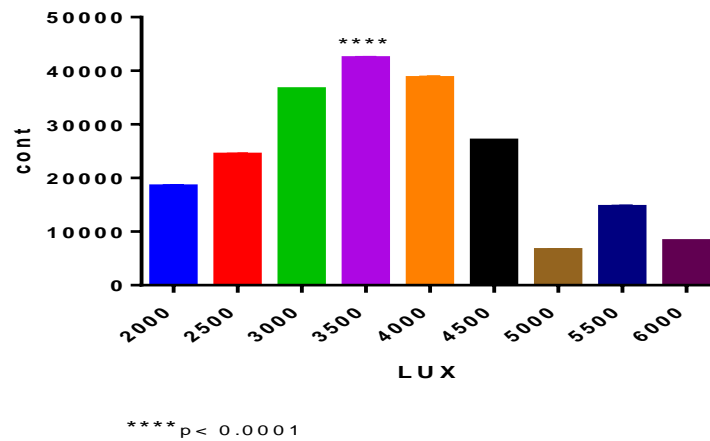


**Figure 8.** The growth optimization of algae with the Tm factor and dry weight.

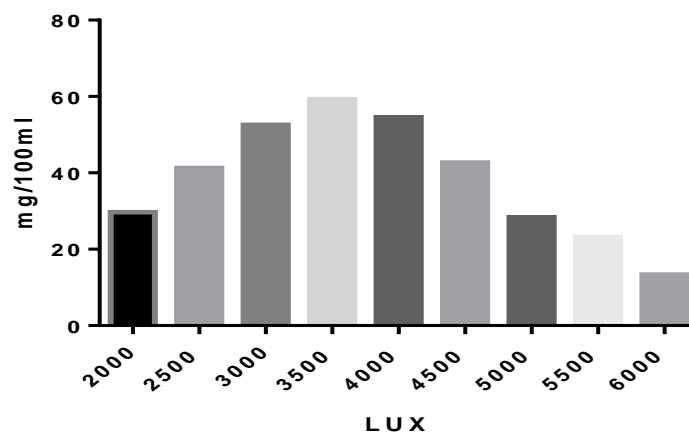
The highest growth optimization was 25°C, with the best cell growth of about 40000 cells. The highest biomass was detected at pH=5.1 and 25°C (57 mg/100 ml). The highest biomass growth was observed at a light intensity of 3500 LUX (60 mg/100 ml), as shown in Figures 9, 10, and 11, which present the results of growth optimization of algae with light intensity factor, cell counting, and dry weight.



**Figure 9.** The growth optimization of algae with the light intensity factor.



**Figure 10.** The growth optimization of algae with the light intensity factor and cell counting.



**Figure 11.** The growth optimization of algae with the light intensity factor and dry weight.

Today, the usage of algae has increased in the agricultural, pharmaceutical, and food industries, and it also has very wide dimensions and modern technology for the production and operation of algae in the world's advanced industrial countries [5]. Fortunately, the spread of algae is remarkable in Iran, and it can supply medicinal raw material producers. Over 250 algae have been identified and applied in Iran [24]. In 1827, Turpin *et al.* isolated *Spirulina* from a freshwater sample [25, 26]. Wittrock and Nordstedt reported the presence near Montevideo [25]. In 1967, *Spirulina* was established as a “wonderful future food source” by the International Association of Applied Microbiology [27, 28]. *Spirulina* growth is mainly controlled by various environmental factors like salts, light, temperature, pH, and nutrients [29, 30]. Scientists and researchers investigated how to optimize and develop industrial cultivation of *Spirulina*. However, the cultural conditions differ in every region of the world, although they have similarities [31, 32]. In this study, we optimized the algae *Spirulina platensis* in different environments (130) and selected the best condition with various conditions such as PH, temperature, light intensity, or dry mass. In this experiment, different environments 130 were tested according to the developmental environment. Media 18 was selected, which includes  $\text{NaHCO}_3$  (7.8 gr),  $\text{K}_2\text{HPO}_4$  (0.5 gr),  $\text{NaNO}_3$  (1.8 gr),  $\text{K}_2\text{SO}_4$  (0.5 gr),  $\text{NaCl}$  (0.5 gr),  $\text{MgSO}_4$  (0.4 gr),  $\text{CaCl}_2$  (0.04 gr),  $\text{FeSO}_4$  (0.4 gr), EDTA (0.08 gr). The best absorption and cell counting were 036.1 nm-34173-cell counting, respectively. Based on this information, media 18 was selected for the following factors: pH ranged from 7 to 8.12, and optical absorption was 86.1 nm at pH 10.5. Cell counting was approximately 209640 cells at pH = 10.5. After that, it was

tested on the dry mass; please see the best dry mass (about 2.49 mg per 100 ml) at pH 10.5. According to statistical data ( $P < 0.0001$ ).

Salt deficiency and increasing amounts cause an imbalance of the cellular ions, resulting in ion toxicity and osmotic stress, leading to retardation of growth [33, 34]. The salt concentration (primarily carbonates and bicarbonates) plays a direct role in *Spirulina* growth [35, 36]. Vincent and Silvester (1979) reported that pH directly affected the physiological properties of algae and the availability of a nutrient [37, 38]. *Spirulina* grew well at pH values between 9 and 11.5 [20]. Light is one of the major challenges in microalga physiology, especially with an increase in biomass [39]. The temperature's role in algae's biochemical reactions makes it one of the most important environmental factors [40]. Therefore, salts, PH, light, and temperature are the four main factors considered for cultivation.

Ogbonda *et al.* (2007) tested *Spirulina* sp. under controlled laboratory conditions (30°C, pH 9), and the highest total crude protein, survival, and cell growth were observed, which was similar to our results [41]. Jimenez *et al.* (2003) showed that the highest growth was at pH 9.5 [42, 43]. Chaiklahan (2012) demonstrated that *Spirulina* sp. had optimal growth at pH 10 and 35°C [44].

The second factor is temperature. The temperature was from 20 to 40 degrees, and optical absorption was being tested. The best absorption at 560 nm was 1.95 at 25°C, and the cell count was 109,376 cells. (Figure 3-8). And after that, it was tested on a dry mass. The best dry mass (about 6.53 in 100 mg/ml) was obtained at a temperature of 25°C, and according to the statistical data ( $P < 0.0001$ ). The best environment was media 18 and pH 10.5, and Tm 25°C. Herrera-Peralta, C. *et al.* (2022) achieved the maximum efficiency of survival and the highest absorption of *Spirulina* sp cells at a temperature of 30°C. Also, he found the most cell growth compared to Tm (35 and 30°C) [45]. Rickmonde (1990) showed that the best and ideal temperature for the growth of *Spirulina* sp was 18 to 30°C [46, 47].

The third factor is light intensity. The severity of the light was tested from 2000 to 6000 Lux, and optical absorption was tested. The best absorption was at 145.2 nm at a light intensity of 3500 Lux, and cell counting was 127071 cells at 3500 Lux. In addition, after that, it was tested on a dry mass. The highest dry mass (about 3.59 mg/100ml) was observed at the highest light intensity (3500 Lux), according to the statistical data ( $P < 0.0001$ ). Pandey *et al.* (2010) reported that the highest dry weight, protein, and chlorophyll were observed at 5000 Lux and pH 9.5 in *Spirulina* sp., which was similar to our study [48, 49]. Sorokin *et al.* (1965) reported that increased light inhibits cell division [50]. The results showed that the best growth conditions were in culture medium 18 (25°C, pH = 10.5, and light intensity = 3500 LUX), and the dry mass obtained was 40 mg per 100 ml, representing the best performance and optimized environmental conditions for *Spirulina platensis* growth.

#### 4. Conclusions

The best growth and optimized environmental conditions were in culture medium 18 (25°C, pH=10.5 and light intensity = 3500 LUX), which were achieved for *Spirulina platensis*, and these optimization conditions can be used for more production.

#### Author Contributions

Conceptualization, N.G.A.; methodology, N.G.A., H.S.; software, A.H.C.; validation, A.H.C.; formal analysis, N.G.A., M.F.; investigation, A.C., M.G., N.G.A.; resources, M.G., A.M.L.;

data curation, A.C., M.F.; writing—original draft preparation, N.G.A.; writing—review and editing, A.C., H.S.; visualization, A.H.C.; supervision, A.C., H.R.S.; project administration, A.M.L.; funding acquisition, A.C., H.R.S. All authors have read and agreed to the published version of the manuscript.

### **Institutional Review Board Statement**

This study involved the cultivation and optimization of growth conditions for the microalga *Spirulina platensis* and did not involve human participants or animals. Therefore, ethical approval for human or animal subjects was not required. The study was conducted in accordance with institutional guidelines and standards at Amiran Oghyanoos Biotechnology Company and collaborating institutes

### **Informed Consent Statement**

Not applicable. This study did not involve human participants or identifiable personal data, and therefore, informed consent was not required

### **Data Availability Statement**

Data supporting the findings of this study are not publicly available due to proprietary restrictions but can be obtained from the corresponding author upon reasonable request.

### **Funding**

This research received no external funding.

### **Acknowledgments**

The authors highly appreciate Isfahan PN University for its support.

### **Conflicts of Interest**

The authors declare no conflict of interest.

### **References**

1. Goswami, R.K.; Mehariya, S.; Karthikeyan, O.P.; Gupta, V.K.; Verma, P. Multifaceted application of microalgal biomass integrated with carbon dioxide reduction and wastewater remediation: A flexible concept for sustainable environment. *J. Clean. Prod.* **2022**, *339*, 130654, <https://doi.org/10.1016/j.jclepro.2022.130654>.
2. Arutselvan, C.; Kumar Seenivasan, H.; Oscar, F.L.; Ramya, G.; Chi, N.T.L.; Pugazhendhi, A.; Thajuddin, N. Review on wastewater treatment by microalgae in different cultivation systems and its importance in biodiesel production. *Fuel* **2022**, *324*, 124623, <https://doi.org/10.1016/j.fuel.2022.124623>.
3. Junior, W.G.M.; Gorgich, M.; Corrêa, P.S.; Martins, A.A.; Mata, T.M.; Caetano, N.S. Microalgae for biotechnological applications: Cultivation, harvesting and biomass processing. *Aquac.* **2020**, *528*, 735562, <https://doi.org/10.1016/j.aquaculture.2020.735562>.
4. Liu, X.; Xie, H.; Roussou, S.; Lindblad, P. Current advances in engineering cyanobacteria and their applications for photosynthetic butanol production. *Curr. Opin. Biotechnol.* **2022**, *73*, 143-150, <https://doi.org/10.1016/j.copbio.2021.07.014>.
5. Rawat, J.; Gupta, P.K.; Pandit, S.; Priya, K.; Agarwal, D.; Pant, M.; Thakur, V.K.; Pande, V. Latest Expansions in Lipid Enhancement of Microalgae for Biodiesel Production: An Update. *Energies* **2022**, *15*, 1550, <https://doi.org/10.3390/en15041550>.

6. Soltani, N.; Latifi, AM.; Alnajar, N.; Dezfulian, M.; Shokarvi, S.; Heydari, M.; Choopani, A. Biochemical and Physiological Characterization of Tree Microalgae spp. as Candidates for Food Supplement. *J. Appl. Biotechnol. Rep.* **2016**, *3*, 377-381.
7. Saggai, A.; Dadamoussa, B.; Djaghoubi, A.; Bissati, S. Production of biomass by *Spirulina* at different groundwater type. Case of Ouargla-Southeast Algeria. *AIP Conf. Proc.* **2016**, 1758, 030016, <https://doi.org/10.1063/1.4959412>.
8. Choopani, A.; Ghalishooyan, M. A Biotechnological Solution for Providing Dietary Supplementation for Naval Personnel in Long and Difficult Missions. *J. Mar. Med.* **2020**, *2*, 150-156, <http://dx.doi.org/10.30491/2.3.150>.
9. Verni, M.; Demarinis, C.; Rizzello, C.G.; Pontonio, E. Bioprocessing to Preserve and Improve Microalgae Nutritional and Functional Potential: Novel Insight and Perspectives. *Foods* **2023**, *12*, 983, <https://doi.org/10.3390/foods12050983>.
10. Lafarga, T.; Ación, G. Microalgae for the Food Industry: From Biomass Production to the Development of Functional Foods. *Foods* **2022**, *11*, 765, <https://doi.org/10.3390/foods11050765>.
11. Ramos-Romero, S.; Torrella, J.R.; Pagès, T.; Viscor, G.; Torres, J.L. Edible Microalgae and Their Bioactive Compounds in the Prevention and Treatment of Metabolic Alterations. *Nutrients* **2021**, *13*, 563, <https://doi.org/10.3390/nu13020563>.
12. Torres-Tiji, Y.; Fields, F.J.; Mayfield, S.P. Microalgae as a future food source. *Biotechnol. Adv.* **2020**, *41*, 107536, <https://doi.org/10.1016/j.biotechadv.2020.107536>.
13. Matufi, F.; Choopani, A. *Spirulina*, food of past, present and future. *Health Biotechnol. Biopharma* **2020**, *3*, 1-20.
14. de Mendonca, H.V.; Assemany, P.; Abreu, M.; Couto, E.; Maciel, A.M.; Duarte, R.L.; Barbosa dos Santos, M.G.; Reis, A. Microalgae in a global world: New solutions for old problems?. *Renew. Energy* **2021**, *165*, 842-862, <https://doi.org/10.1016/j.renene.2020.11.014>.
15. Perković, L.; Djedović, E.; Vujović, T.; Baković, M.; Paradžik, T.; Čož-Rakovac, R. Biotechnological Enhancement of Probiotics through Co-Cultivation with Algae: Future or a Trend?. *Mar. Drugs* **2022**, *20*, 142, <https://doi.org/10.3390/md20020142>.
16. Peter, A.P.; Koyande, A.K.; Chew, K.W.; Ho, S.-H.; Chen, W.-H.; Chang, J.-S.; Krishnamoorthy, R.; Banat, F.; Show, P.L. Continuous cultivation of microalgae in photobioreactors as a source of renewable energy: Current status and future challenges. *Renew. Sustain. Energy Rev.* **2022**, *154*, 111852, <https://doi.org/10.1016/j.rser.2021.111852>.
17. Zhu, J.; Rong, J.; Zong, B. Factors in mass cultivation of microalgae for biodiesel. *Chin. J. Catal.* **2013**, *34*, 80-100, [https://doi.org/10.1016/S1872-2067\(11\)60497-X](https://doi.org/10.1016/S1872-2067(11)60497-X).
18. Slade, R.; Bauen, A. Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. *Biomass Bioenergy* **2013**, *53*, 29-38, <https://doi.org/10.1016/j.biombioe.2012.12.019>.
19. Demirbas, M.F. Biofuels from algae for sustainable development. *Appl. Energy* **2011**, *88*, 3473-3480, <https://doi.org/10.1016/j.apenergy.2011.01.059>.
20. Hamidi, M.; Mohammadi, A.; Mashhadi, H.; Mahmoudnia, F. Evaluation of effective environmental parameters on lipid, protein and beta-carotene production in *Spirulina platensis* microalga. *Results Eng.* **2023**, *18*, 101102, <https://doi.org/10.1016/j.rineng.2023.101102>.
21. Choopani, A.; Fazilati, M.; Latifi, A.M.; Salavati, H.; Khodabakhshi, M.R.; Fadaie, M. An Efficient Method for Extraction and Enrichment of  $\gamma$ -Linolenic Acid (GLA) from *Spirulina*. *Lett. Appl. NanoBioScience* **2022**, *11*, 3166-3174, <https://doi.org/10.33263/BRIAC111.31663174>.
22. Zhu, C.J.; Lee, Y.K. Determination of biomass dry weight of marine microalgae. *J. Appl. Phycol.* **1997**, *9*, 189-194, <https://doi.org/10.1023/A:1007914806640>.
23. Yazdi, H.; Karami, A.; Babavalian, H.; Mirhosseini, S.A.; Tebyanian, H. The Effects of some Physicochemical Stresses on *Escherichia coli* O157: H7 as Clinical Pathogenic Bacteria. *Int. J. Agric. Biol.* **2016**, *18*, 1237-1241, <https://doi.org/10.17957/ijab/15.0237>.
24. Sohrabi Pour, J.; Rabii, R. NEW RECORDS OF ALGAE FOR PERSIAN GULF AND FLORA OF IRAN. *Iran J. Bot.* **2015**, *7*, 95-117, <https://doi.org/10.22092/ijb.2015.103483>.
25. Wittrock, V.; Nordstedt, C. H. Report on the presence of *Spirulina* near Montevideo. Botanical Survey Publications, **1844**, pp. 125-130.
26. Zhang, F.; Man, Y.B.; Mo, W.Y.; Wong, M.H. Application of *Spirulina* in aquaculture: a review on wastewater treatment and fish growth. *Rev. Aquac.* **2020**, *12*, 582-599, <https://doi.org/10.1111/raq.12341>.

27. Pandey, J.P.; Tiwari, A.; Mishra, G.; Mishra, R.M. Role of *Spirulina maxima* in the Control of Blood Glucose Levels and Body Weight in Streptozotocin induced Diabetic Male Wistar rats. *J. Algal Biomass Utln.* **2011**, *2*, 35-37.
28. Vrenna, M.; Peruccio, P.P.; Liu, X.; Zhong, F.; Sun, Y. Microalgae as Future Superfoods: Fostering Adoption through Practice-Based Design Research. *Sustainability* **2021**, *13*, 2848, <https://doi.org/10.3390/su13052848>.
29. Jafarpour, R.; Vahdat, S.; Asadi, F.; Dardashti, H.K.; Zarei, B.; Dadkhah, A. Investigating the growth rate of *Dunaliella tertiolecta* in the optimum N:P ratio in mono and mixed cultures. *J. Appl. Phycol.* **2023**, *35*, 1553-1563, <https://doi.org/10.1007/s10811-023-02971-8>.
30. Juneja, A.; Ceballos, R.M.; Murthy, G.S. Effects of Environmental Factors and Nutrient Availability on the Biochemical Composition of Algae for Biofuels Production: A Review. *Energies* **2013**, *6*, 4607-4638, <https://doi.org/10.3390/en6094607>.
31. Salehian Lenji, N.; Jahadi, M.; Ataabadi, M. Optimization of spirulina platensis and natural pigment production by response surfaces production. *J. Food Sci. Technol.* **2022**, *19*, 343-355, <http://doi.org/10.22034/FSCT.19.131.343>.
32. Belal, E.B.; Khalafalla, M.M.E.; El-Hais, A.M.A. Use of Spirulina (*Arthrospira fusiformis*) for promoting growth of Nile Tilapia fingerlings. *Afr. J. Microbiol. Res.* **2012**, *6*, 6423-6431.
33. Madkour, F.F.; Kamil, A.E.-W.; Nasr, H.S. Production and nutritive value of *Spirulina platensis* in reduced cost media. *Egypt. J. Aquat. Res.* **2012**, *38*, 51-57, <https://doi.org/10.1016/j.ejar.2012.09.003>.
34. Lu, W.; Alam, M.A.; Liu, S.; Xu, J.; Saldivar, R.P. Critical processes and variables in microalgae biomass production coupled with bioremediation of nutrients and CO<sub>2</sub> from livestock farms: A review. *Sci. Total Environ.* **2020**, *716*, 135247, <https://doi.org/10.1016/j.scitotenv.2019.135247>.
35. 'Kol'chugina, I.B.; Markarova, E.N. Role of Sodium Ions and Their Uptake by Cells of Cultured Blue-Green Algae, *Spirulina platensis* and *Spirulina maxima*. *Microbiology* **2005**, *74*, 646-649, <https://doi.org/10.1007/s11021-005-0118-4>.
36. Matos, Â.P.; da Silva, T.; Sant'Anna, E.S. The Feasibility of Using Inland Desalination Concentrate (DC) as an Alternative Substrate for *Spirulina platensis* Mass Cultivation. *Waste Biomass Valor.* **2021**, *12*, 3193-3203, <https://doi.org/10.1007/s12649-020-01233-9>.
37. Vincent, W.F.; Silvester, W.B. Growth of blue-green algae in the Manukau (New Zealand) oxidation ponds—II. Experimental studies on algal interaction. *Water Res* **1979**, *13*(8), 717-23, [https://doi.org/10.1016/0043-1354\(79\)90234-3](https://doi.org/10.1016/0043-1354(79)90234-3).
38. Hao, B.; Wu, H.; You, J.; Xing, W.; Cai, Y. Biomass and physiological responses of green algae and diatoms to nutrient availability differ between the presence and absence of macrophytes. *Ecol. Indic.* **2021**, *129*, 107987, <https://doi.org/10.1016/j.ecolind.2021.107987>.
39. Barbosa, M.J.; Janssen, M.; Ham, N.; Tramper, J.; Wijffels, R.H. Microalgae cultivation in air-lift reactors: Modeling biomass yield and growth rate as a function of mixing frequency. *Biotechnol. Bioeng* **2003**, *82*, 170-179, <https://doi.org/10.1002/bit.10563>.
40. Hu, Q. Environmental Effects on Cell Composition. In Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second Edition, Richmond, A.; Emeritus; Hu, Q., Eds.; **2013**, 114-122, <https://doi.org/10.1002/9781118567166.ch7>.
41. Ogbonda, K.H.; Aminigo, R.E.; Abu, G.O. Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina* sp. *Bioresour. Technol.* **2007**, *98*, 2207-2211, <https://doi.org/10.1016/j.biortech.2006.08.028>.
42. Jiménez, C.; Cossí, B.R.; Niell, F.X. Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: a predictive model of algal yield. *Aquac.* **2003**, *221*, 331-345, [https://doi.org/10.1016/S0044-8486\(03\)00123-6](https://doi.org/10.1016/S0044-8486(03)00123-6).
43. Soni, R.A.; Sudhakar, K.; Rana, R.S. *Spirulina*—From growth to nutritional product: A review. *Trends Food Sci. Technol.* **2017**, *69*, 157-171, <https://doi.org/10.1016/j.tifs.2017.09.010>.
44. Chaiklahan, R.; Chirasuwan, N.; Bunnag, B. Stability of phycocyanin extracted from *Spirulina* sp.: Influence of temperature, pH and preservatives. *Process Biochem.* **2012**, *47*, 659-664, <https://doi.org/10.1016/j.procbio.2012.01.010>.
45. Herrera-Peralta, C.; Maldonado-Pereira, L.; Cantú-Lozano, D.; Medina-Meza, I.G. Mixotrophic cultivation boost nutraceutical content of *Arthrospira (Spirulina) platensis*. *agriRxiv* **2022**, *2022*, 20220173394 <https://doi.org/10.31220/agriRxiv.2022.00139>.

46. Gao, S.; Pittman, K.; Edmundson, S.; Huesemann, M.; Greer, M.; Louie, W.; Chen, P.; Nobles, D.; Benemann, K.; Crowe, B. A newly isolated alkaliphilic cyanobacterium for biomass production with direct air CO<sub>2</sub> capture. *J. CO<sub>2</sub> Util.* **2023**, *69*, 102399, <https://doi.org/10.1016/j.jcou.2023.102399>.
47. Shi, W.-q.; Li, S.-d.; Li, G.-r.; Wang, W.-h.; Chen, Q.-x.; Li, Y.-q.; Ling, X.-w. Investigation of main factors affecting the growth rate of *Spirulina*. *Optik* **2016**, *127*, 6688-6694, <https://doi.org/10.1016/j.ijleo.2016.04.125>.
48. de Jesús, A.; Roldán, M.; Paloma, B.; Lozano, G. Growth of *Spirulina Maxima* in an Optimized Concentric Internal Tube Photobioreactor. *Iran J. Biotechnol.* **2022**, *20*, e2985, <http://doi.org/10.30498/ijb.2022.273070.2985>.
49. Nosratimovafagh, A.; Fereidouni, A.E.; Krujatz, F. Modeling and Optimizing the Effect of Light Color, Sodium Chloride and Glucose Concentration on Biomass Production and the Quality of *Arthrospira platensis* Using Response Surface Methodology (RSM). *Life* **2022**, *12*, 371, <https://doi.org/10.3390/life12030371>.
50. Hussin, A.A.; To, S.W.; Sani, M.H.; Amin, M.F.M.; Kamaroddin, M.F. Optimisation and growth kinetic analysis of Microalgae, *Arthrospira platensis* in 2-L Photobioreactors. *IOP Conf. Ser.: Earth Environ. Sci.* **2021**, *842*, 012036, <http://doi.org/10.1088/1755-1315/842/1/012036>.

### **Publisher's Note & Disclaimer**

The statements, opinions, and data presented in this publication are solely those of the individual author(s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.