Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 13, Issue 4, 2024, 151
https://doi.org/10.33263/LIANBS134.151

Evaluation of *Spirulina platensis* Growth Factors for High-Efficiency Biomass Production

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Scopus Author ID 1888779
Received: 13.05.2023; Accepted: 11.08.2023; Published: 28.07.2024

Abstract: *Spirulina platensis* is an important microalga cultivated commercially in many tropical countries and sub-tropical and temperate regions to feed humans and animals**.** The cost of the culture medium is containing 15 to 20 percent of its 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 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 the best growth conditions were in culture medium No: 18, 25°C, pH=10.5, and light intensity = 3500 LUX), and the dry mass was obtained 40mg per 100ml. This study achieved the best performance and optimized environmental conditions for *Spirulina platensis*.

Keywords: s*pirulina platensis*; biomass; culture; optimization.

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) have been cultured and harvested annually for different aims [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 finding and ing the features of valuable compounds that have medicinal properties and are produced by photosynthetic organisms [4]. Algae has been considered a powerful organism 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 bulky volumes of biological mass with finite amounts of valuable compounds at a 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* produces about 2000 to 3000 tons per year, and the majority of its production is planted in the outdoor pool. The production of algae has been done 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 pastille [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 big scale since 1940 [15, 16]. The main effort is to reach the appropriate balance between different factors affecting the growth of the Lake, such as light, temperature, nutrients, PH, and mixing. Light should be a restricted factor in achieving maximum biomass [15]. Theoretical maximum conversion efficiency is 6.6% for optical energy, but an acceptable level is lower than this rate; for example, a high production rate of *Spirulina* converts solar energy to 1% efficiency. Some nutrients are necessary for algae growth in culture media. These materials can include nutrients, trace elements, and vitamins and can be variable according to the target species and the typical products. Biomass products are increased by adding an organic carbon source, and the types of carbon sources used depend on the 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, these needs may be different according to the final product, such as lipids and 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, in The Center of Shahid Beheshti University identified by morphological, physiological, and biological laboratory keys. And cultivated with different volumes of Parameters. One hundred thirty different cultures' media are used for culturing. In addition, media cultures randomly selected from nine materials include $NaHCO_3$, K_2HPO_4, $NaNO_3$, K_2SO_4, NaCl, $MgSO_4$, $CaCl_2$, $FeSO_4$, and EDTA. A UV lamp was used to sterilize the culture room for about half an hour. Arlene was washed with distilled water and sterile in Avon at 100°C to dry for one hour. For mass culture, 10ml of pure algae containing 9×10^8 (Cell/ml) were inoculated was on to Arlene 250ml containing culture media sterile 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. One hundred thirty 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,

the pH gradient was tested with 7-8 and 12 for a month OD, and 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 variable quantities of light (light intensity), lighting time, and darkness were studied. 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 number 18 had the highest absorption among 130 cultures in the first, second, third, and fourth weeks, which is shown in Figure 1.

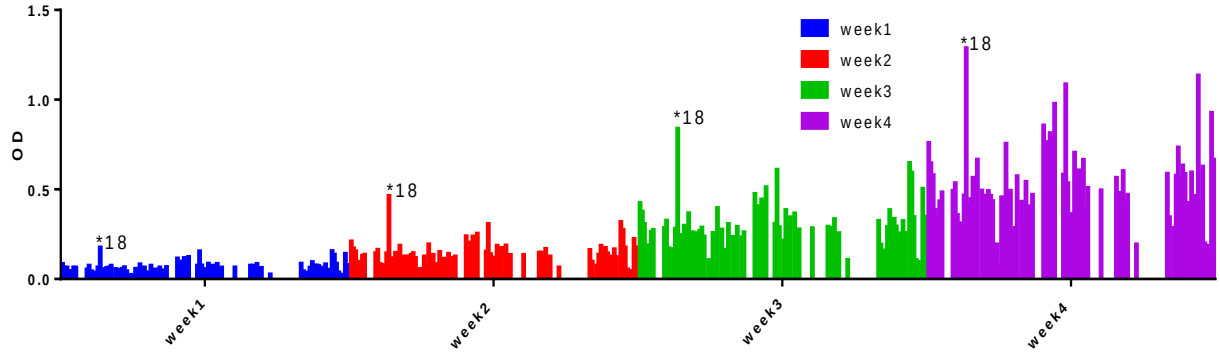

Figure 1. Absorption OD of Culture number 18.

Growth optimization was not detected in the first week, but this growth optimization gradually increased, and the highest growth optimization was in the fourth week. The results of the growth optimization of Culture number 18 are shown in Figure 2.

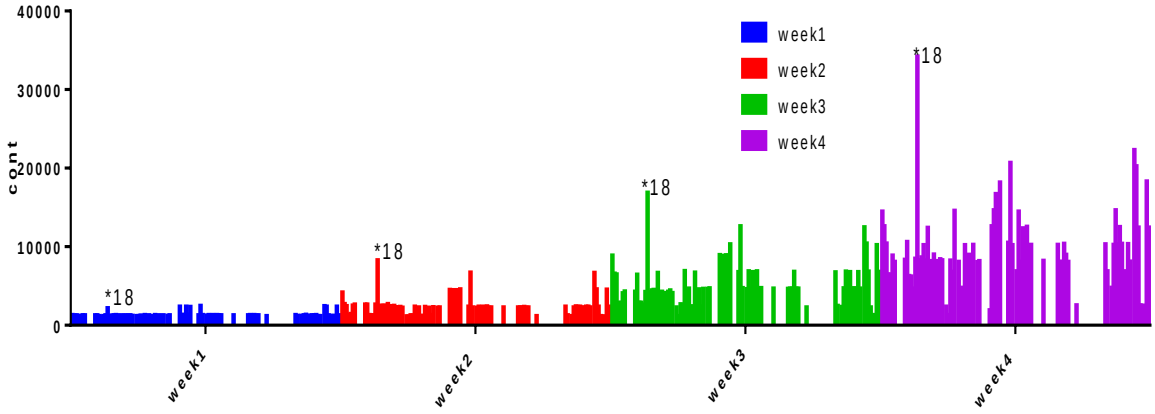

Figure 2. Cell counts of Culture number 18.

Figure S3 shows that the best pH for growth optimization was 10.5 (over 40mg/100ml). The results of the growth optimization of algae with pH factor, cell counting, and suffering (7-8.12), as well as the preparation of dry weight, are shown in Figures 4 and 5.

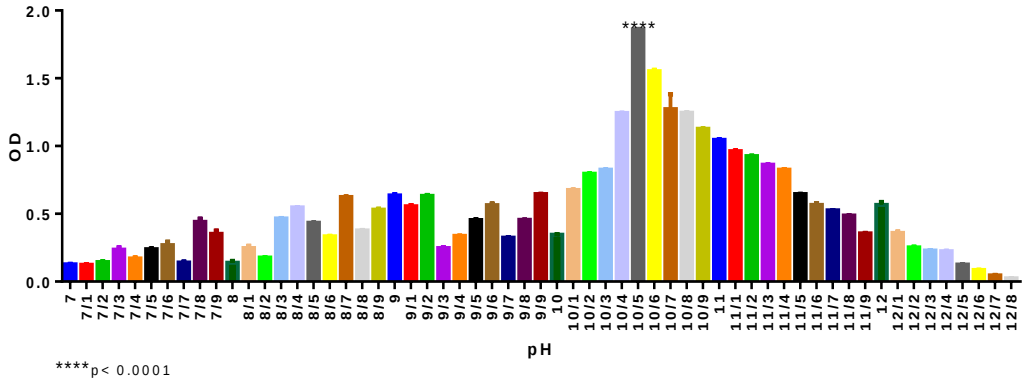

Figure 3. The algae growth with the pH factor.

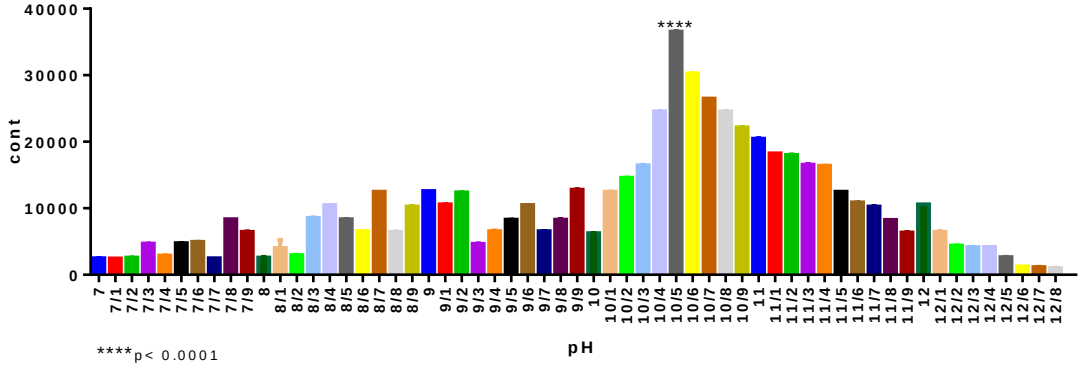

Figure 4. The algae growth with pH factor and cell counting.

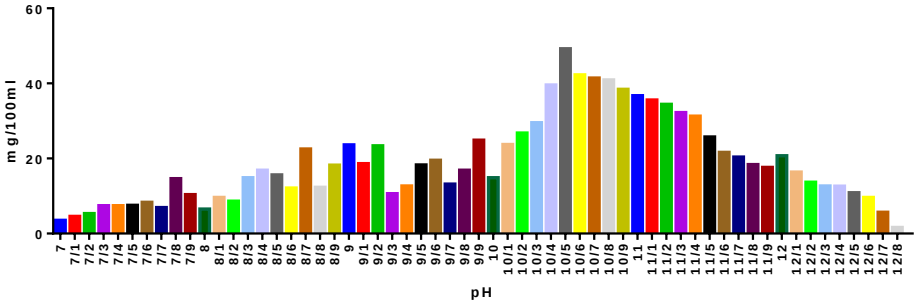

Figure 5. The algae growth with the pH factor in suffering (7-8.12) and the preparation of dry weight

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

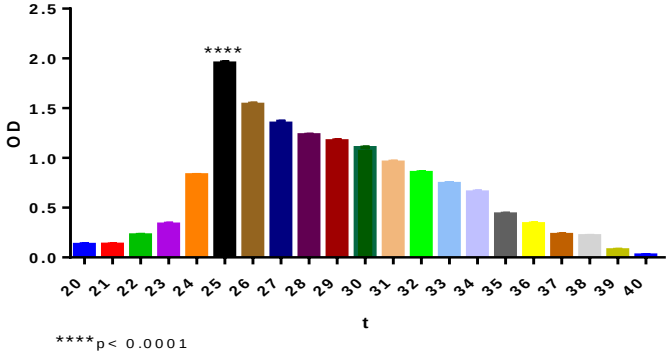

Figure 6. The growth optimization of algae with Tm factor.

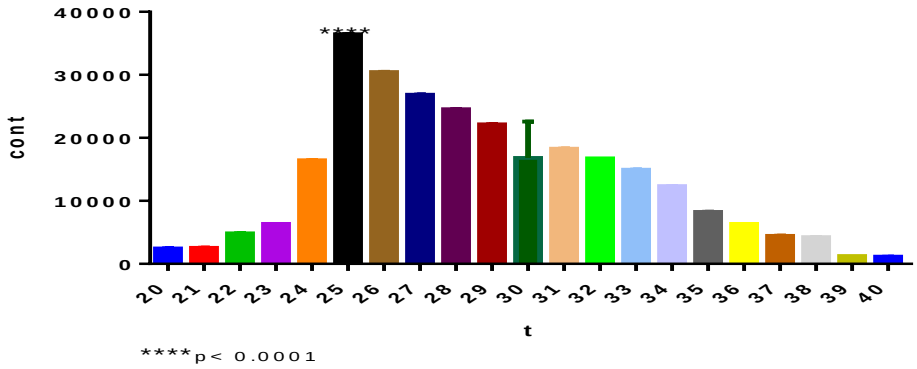

Figure 7. The growth optimization of algae with Tm factor and cell counting.

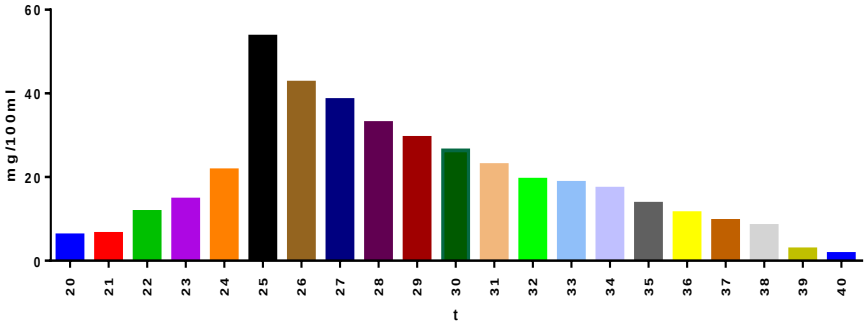

Figure 8. The growth optimization of algae with Tm factor and dry weight.

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

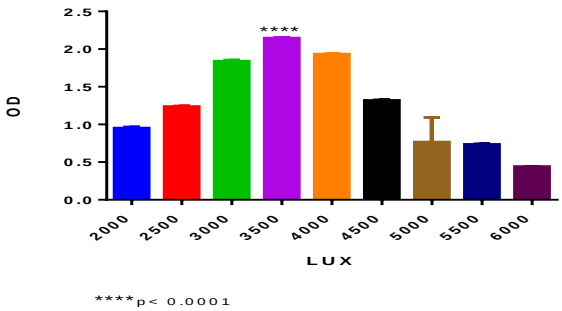

Figure 9. The growth optimization of algae with light intensity factor.

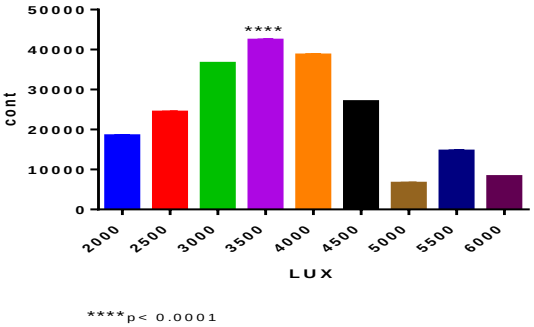

Figure 10. The growth optimization of algae with light intensity factor and cell counting.

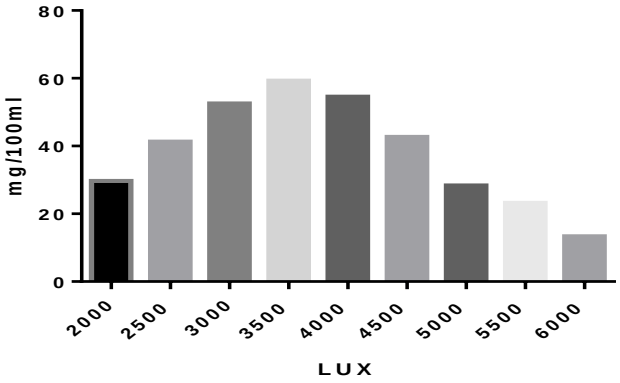

Figure 11. The growth optimization of algae with light intensity factor and dry weight.

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, P.J. Turpin 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 to optimize and develop industrial cultivation of *spirulina*. However, this proves that the cultural conditions differ in every region of the world, although they have similarities [31, 32]. In this study, we optimized the algae *Spirulina platinis* in different environments (130) and selected the best conditions with various conditions such as PH, temperature, light intensity, or dry mass. In this experiment, different environments 130 (according to Table 2-1) were tested according to the developmental environment, and media 18 was selected which its media includes $NaHCO_3$(7.8 gr), K_2HPO_4 (0.5gr), $NaNO_3$ (1.8gr), K_2SO_4 (0.5gr), Nacl (0.5gr), $MgSO_4$ (0.4gr), $CaCl_2$ (0.04gr), $FeSO_4$ (0.4gr), EDTA(0.08gr). The best absorption and cell counting were 036.1 nm-34173-cell counting, respectively. Based on this information, media 18 was selected based on the factors described below: pH was tested from 7 to 8.12, and optical absorption was 86.1nm 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 ($P < 0.0001$).

Salts deficiency and increasing amounts cause an imbalance of the cellular ions a, resulting in ion toxicity and osmotic stress, leading to retardation of growth [33, 34]. The salt concentration (mostly carbonates and bicarbonates) plays a direct role in the growth of *Spirulina* [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 increased biomass [39]. The temperature's role in the biochemical reactions of algae 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. in a controlled laboratory condition (30℃, pH 9), and the highest total amount of crude protein, survival, and growth of the cells were absorbed, 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 the best growth conditions 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), After that, it was tested on a dry mass .The best dry mass (about 6.53 in 100mg/ml) at a temperature of 25°C and according to the statistical data ($P < 0.0001$). The best environment was media 18 pH 10.5, and Tm 25°C. 45. 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 145.2nm at 3500 lux light intensity, and cell counting was 127071 cells at 3500 Lux. In addition, after that, it was tested on a dry mass. The best dry mass (about 3.59mg/100ml) was in the intensity of the light (3500 Lux) according to the statistical data ($P < 0.0001$). Pandey *et al*. (2010) reported that the greatest dry weight, protein, and chlorophyll was 5000 Lux and pH 9.5 in Spirulina sp, similar to our study [48, 49]. Sorokin *et al*. (1965) reported that the increase of light causes the inhibition of cell division [50]. The results showed the best growth conditions was in culture medium 18 (25°C pH=10.5 and light intensity = 3500 LUX) and the dry mass obtained 40mg per 100ml which these conditions were 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.

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.

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