Iranian Journal of Fisheries Sciences 20(2) 475-489 2021
DOI: 10.22092/ijfs.2021.351071.0

Research Article

Cultivations of *Arthrospira maxima* (Spirulina) using ammonium sulfate and sodium nitrate as an alternative nitrogen sources

Mirhosseini N.[1]; Davarnejad R.[1*]; Hallajisani A.[2]; Cano-Europa E.[3*]; Tavakoli O.[4]; Franco-Colín M.[2]; Blas-Valdivia V.[5]

Received: July 2020 Accepted: December 2020

Abstract

Arthrospira (Spirulina) has been considered as an attractive microalgae in all aspects of human life including medicine, cosmetics, and food. Nitrogen source is an important cost-saving factor in large-scale cultivation. In the present study, the cultivation of *S. maxima* was studied by replacing the basic-nitrogen source of Zarrouk's medium (2.5 gL^{-1}) with concentration ranges of 0-10 gL^{-1} for sodium nitrate and 0-5 gL^{-1} for ammonium sulfate in terms of biomass and phycobiliproteins production. Biomass and phycobiliprotein growth of different nitrogen sources have shown different effects on growth. The changes in the amount of cell dry weight as a function of sodium nitrate did not show significant changes relating to its concentration. In case of ammonium sulfate, the cell dry weight of *S. maxima* without nitrogen source was 0.835 gL^{-1} during five days of cultivation. Moreover, phycocyanin and allophycocyanin contents were 0.053 and 0.072 mgL^{-1}, respectively, while phycobiliproteins content and cell dry weight were decreased by increasing further concentration. There was a significant difference among the culture mediums containing ammonium sulfate and without nitrogen source in terms of concentration of biomass and phycobiliprotein. The highest and lowest results for cell dry weight and phycobiliprotein production were obtained from the treatment with nitrogen starvation and 5 gL^{-1} ammonium sulfate, respectively. Finally, nitrogen starvation was proved as a feasible way to grow and could be good candidate for biomass growth and phycobiliprotein.

Keywords: *Arthrospira maxima*, Nitrogen sources, Ammonium sulfate, Sodium nitrate

1-Department of Chemical Engineering, Faculty of Engineering, Arak University, Arak, 38156-8-8349 Iran
2-Biofuel Laboratory, Caspian Faculty of Engineering, College of Engineering, University of Tehran, Tehran, Iran
3-Laboratorio de Metabolismo I, Departamento de Fisiología, Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional. Av. Wilfrido Massieu s/n, Esq. Manuel L. Stampa, Colonia Unidad Profesional Adolfo López Mateos, Delegación Gustavo A. Madero, Código Postal 07738 Ciudad de México, Mexico
4-School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran.
5-Laboratorio de Neurobiologıa, Departamento de Fisiología, Escuela Nacional de Ciencias Biologicas, Instituto Politecnico Nacional, Código Postal 07738 Ciudad de México, Mexico.
*Corresponding authors' Email: r-davarnejad@araku.ac.ir; edgarcanoeuropa@yahoo.com.mx; ecanoe@ipn.mx

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

Introduction

Natural pigments are current interest in the market because of their color functions and physiological activity compared with synthetic colorant agents (Sigurdson *et al*., 2017). Furthermore, there is a massive demand for natural coloring agents in industries like food, drug, and cosmetics (Clydesdale, 1993; Wissgott and Bortlik, 1996). Most recently, the feasibility of natural pigments extraction from microalga has attracted considerable interest worldwide, while the use of most or all synthetic colorants has been banned by FDA (Kevin B. Hicks *et al*., 1992; Beatriz *et al*., 2015).

Among these pigments, applications of phycocyanin and allophycocyanin as a natural color from alga have massive values in nutraceutical, cosmetics, and pharmaceutical industries owing to their health benefits (Kuddus *et al*., 2013). Currently, Spirulina is the widely available microalga source that contains a high value of phycocyanin and allophycocyanin pigments (Park *et al*., 2018). Among various species of this algae, *S. platensis* and *S. maxima* are the two most important species (Sánchez *et al*., 2003). *S. maxima* is a photosynthetic, filamentous, and multicellular blue-green cyanobacterium with a long history of use as food (Ashton Acton, 2013). Traditionally, *S. maxima* was harvested from the Texcoco lake for human consumption (Vo *et al*., 2015).

According to researchers, Spirulina production is mainly depending on nutrient availability, temperature, and light (Carvalho and Malcata, 2003; Kumar *et al*., 2011). Previously, a wide number of media have been developed for the Spirulina biomass growth like modified Zarrouk (Rajasekaran *et al*., 2016), revised medium (RM6) (Raoof *et al*., 2006), CFTRI, JPJM (Salunke *et al*., 2016), Convy, f/2, BG-11 (Dineshkumar *et al*., 2016), and Bangladesh medium (Khatun *et al*., 1970). Moreover, Zarrouk's medium has been known as the conventional standard medium for Spirulina culture (Zarrouk, 1966; Raoof *et al*., 2006).

Nitrogen and carbon sources are the most cost-effective factor in the growth and pigment production of Spirulina (Soletto *et al*., 2005; Çelekli and Yavuzatmaca, 2009). Sodium nitrate is the common nitrogen source that has been used for Spirulina cultivation (Cost *et al*., 2001). The replacement of basic nitrogen sources of culture medium with cheaper materials such as urea, ammonium sulfate, and ammonium chloride have been previously studied in *S. platensis* cultivation (Carvalho *et al*., 2004; Bezerra *et al*., 2008; Ferreira *et al*., 2010; Avila-Leon *et al*., 2012;; Matsudo *et al*., 2012).

To the best of our knowledge, biomass and pigment production of *S. maxima* has not been widely studied in evaluating the effect of nitrogen sources. Therefore, the present study aimed to evaluate the effect of two different nitrogen sources (sodium nitrate and nitrogen sulfate) at different concentrations on the specific growth

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

rate, cell dry weight, and phycobiliproteins production.

Materials and methods

Microorganism and Culture condition:

S. maxima strain CIB79 was obtained from National Polytechnic University, (IPN, Mexico). The experiment was carried out at laboratory temperature using white fluorescent lamps (4,500 lux). The cultures were incubated under continuous illumination and aeration with air pump AC-9602 (RESUN, Mexico) during the process of growth. We studied the influence of two different nitrogen sources (i.e., sodium nitrate and ammonium sulfate) at various concentrations and cultivation time (Table 1) to estimate growth and phycobiliproteins production in the pure culture of Zarrouk's medium. Nitrogen sources were replaced by the basic-nitrogen source of Zarrouk's medium (2.5 gL^{-1}). Nitrogen sources were bath added in 125 ml flask with 40 ml of *S. maxima* stock (Initial $OD_{674} \cong$ 0.4) during 8 days of cultivation. All experimental cultures were set up in triplicate. The experimental data was obtained as the mean ± SEM value for triplicate cultures in all treatments. The pH was monitored by a pH21 pH/mV meter (HANNA model).

Table 1: Different nitrogen sources and their concentrations.

Treatment No.	Nitrogen source	Nitrate source concentration	Unit	Cultivation time (day)
1	Sodium Nitrate	0	gL^{-1}	8
2		1.25		
3		2.5		
4		5		
5		10		
6	Ammonium Sulfate	0	gL^{-1}	5
7		1.25		
8		2.5		
9		5		

Estimation of biomass growth:

The optical density was determined with a Multiscan Go spectrophotometer (Thermo SCIENTIFIC, England) by measuring the absorbance of the medium at a provided wavelength. To measure the biomass and phycobiliproteins production, the cell dry weight of *S. maxima* was calculated by the equation of Y (gL^{-1}) = 0.58X-0.0201 (correlation coefficient (r) = 0.997), where Y is the cell dry weight of biomass and X is the optical density at 674 nm. We took the samples of cultures to analyze growth and phycobiliproteins contents at specific time intervals. The pigment concentrations of phycocyanin (PC) and allophycocyanin (APC) were calculated using the following equations by Bennett and Bogorad (Bennett and Bogobad, 1973).

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

$$PC\ (gL^{-1}) = (A_{620} - 0.474\,A_{652})/5.34 \quad (1)$$

$$APC\ (gL^{-1}) = (A_{652} - 0.208\,A_{620})/5.09 \quad (2)$$

Where A_{620} *and* A_{652} are absorbance at the wavelength of 620 and 652, respectively.

Furthermore, the maximum specific growth rate (μ_{max}) and the minimum doubling time (t_d) were determined by nonlinear regression of the logarithmic growth phase by plotting cell dry weight versus time using Wolfram Mathematica software version 11.3. Moreover, the nitrogen cell conversion factor of *S. maxima* in different treatments was calculated using the procedure of Danesi *et al*. (2011).

$$Y_{X/N} = (X_m - X_i).V/N_t \quad (3)$$

Where:

$Y_{X/N}$: Nitrogen-cell conversion (gg^{-1})

X_m: Maximum dry weight (gl^{-1})

X_i: Initial cell dry weight (gl^{-1})

V: The total volume of cultivation (L)

N_t: The total quantity of added nitrogen (g)

All graphic designs and pigment calculations in this study were performed using the GraphPad Prism 8 software.

Phycobiliproteins Isolation:

The cell walls were ruptured using repeated five freezing and thawing cycles for phycobiliproteins isolation (Sarada *et al*., 1999). Also, the samples were frozen at -20ºC for 1 h and then were thawed at room temperature for 45 min. After freezing and thawing the samples up to five cycles, they were centrifuged (The Velocity 14/14 R Refrigerated centrifuge, China) at 10,000 rpm for 10 min at 4ºC, where the supernatant was collected for phycobiliproteins determination.

Results

The impact of two different nitrogen sources was studied by replacing the basic-nitrogen source of Zarrouk's medium (2.5 gL^{-1}) with different concentration of sodium nitrate and ammonium sulfate. Fig. 1 shows the changes in biomass growth by cell dry weight (panel A and B) and phycobiliproteins pigment (panel C and D) during the 8 days of cultivation time at different sodium nitrate concentration. Changes of cell dry weight (i.e., 1.209, 1.200, 1.010, 1.001, 0.992) gL^{-1}) were monitored by increasing the concentration of sodium nitrate from 0 to 10 gL^{-1} (Fig. 1, panel B) at the final day of cultivation. Also, it was observed that the highest biomass growth (1.209 gL^{-1}) occurred in the nitrate-free medium. The cell dry weight was decreased with an increase in the amount of nitrogen in the culture medium. Although cell dry weight did not show significant changes related to concentration, even this smallest possible change is very important to reduce the production cost of scaling up. The highest phycocyanin and allophycocyanin production (0.066 and 0.088 mg/L) also occurred in the medium with sodium nitrate starvation (Fig. 1, panels C and D).

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

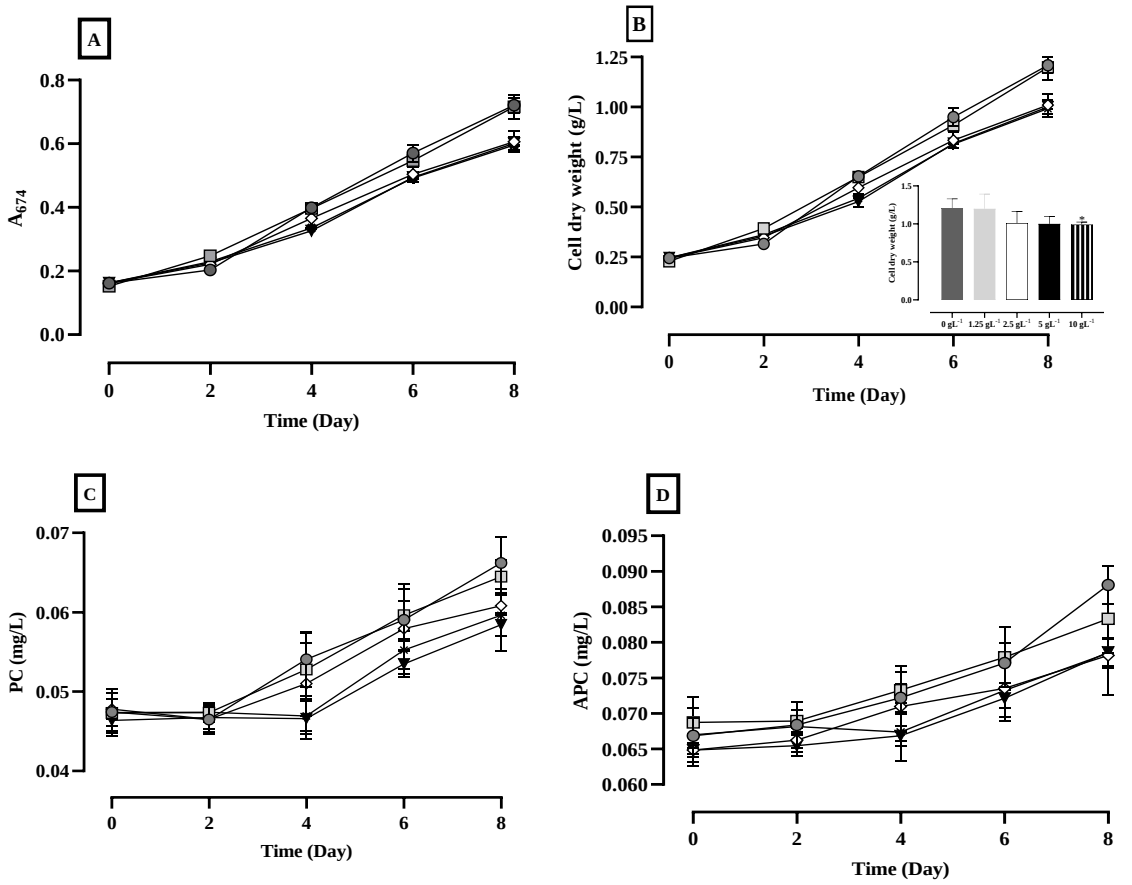

Figure 1: The effect of sodium nitrate concentrations on cellular growth (panel A), cell dry weight (panel B), phycocyanin (panel C) and allophycocyanin content (panel D) during cultivation time. (●: 0 mM, ■: 1.25 gL^{-1}mM, ◊: 30 mM, ▼: 60 mM, ⁎ 120 mM). Values are the mean ± SD of three replicates.

Treatment 5 showed a negligible change compared to treatments 3 and 4. Fig. 2 represents the effect of sulfate ammonium at the different concentration on biomass growth by cell dry weight (panels A and B) and phycobiliproteins pigment (panels C and D) during the cultivation time. In this case, the experiment was stopped due to a decrease in microalgae growth on day 5 when the medium culture was supplemented with ammonium sulfate. Although increasing the concentration of sodium nitrate do not dramatically change the accumulation of phycobiliprotein.

Allophycocyanin content was about 0.072, 0.044, 0.035, and 0.026 mgL^{-1} when cultured in the growth medium treatment 6, treatment 7, treatment 8, and treatment 9, respectively (Fig. 2, panels C and D).

Moreover, the highest and lowest phycocyanin reductions were observed about 79% and 44% after 5 days of cultivation in culture medium treatments 9 and 7, respectively.

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

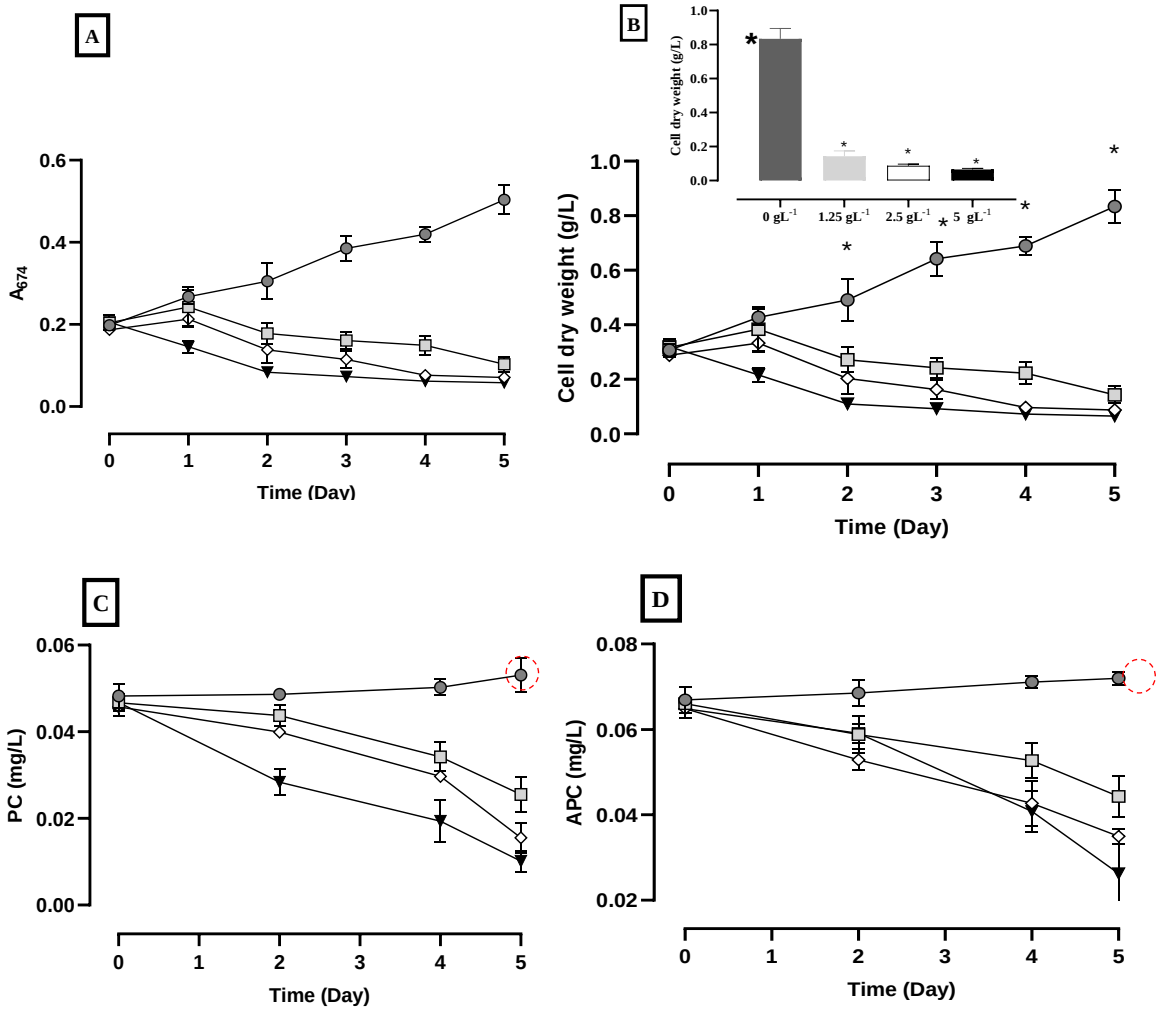

Figure 2: The effect of sulfate ammonium concentration on cellular growth (panel A), cell dry weight (panel B), phycocyanin (panel C) and allophycocyanin content (panel D) during cultivation time (●: 0 gL^{-1}, ■: 1.25 gL^{-1}, ◊: 2.5 gL^{-1}, ▼: 5 gL^{-1}). Values are the mean ± SD of three replicates.

On the basis of the obtained results, it is clear that the changes in biomass growth could influence phycobiliprotein content. Fig. 3 exhibits the effect of sulfate ammonium at different concentrations by changing in medium color at several time-point during cultivation time. The color of matured *S. maxima* in treatment 6 altered from light to dark green (flask with nitrate-free), while it could not grow in other treatments of this group. There was a significant difference among the cultures containing ammonium sulfate and without nitrogen source in terms of concentration of biomass and phycobiliprotein.

The dead cell of *S. maxima* was recognized in treatment 9 when the color of the culture medium was suddenly changed from green light to pale yellow and decolorized cell on the fifth day of cultivation.

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

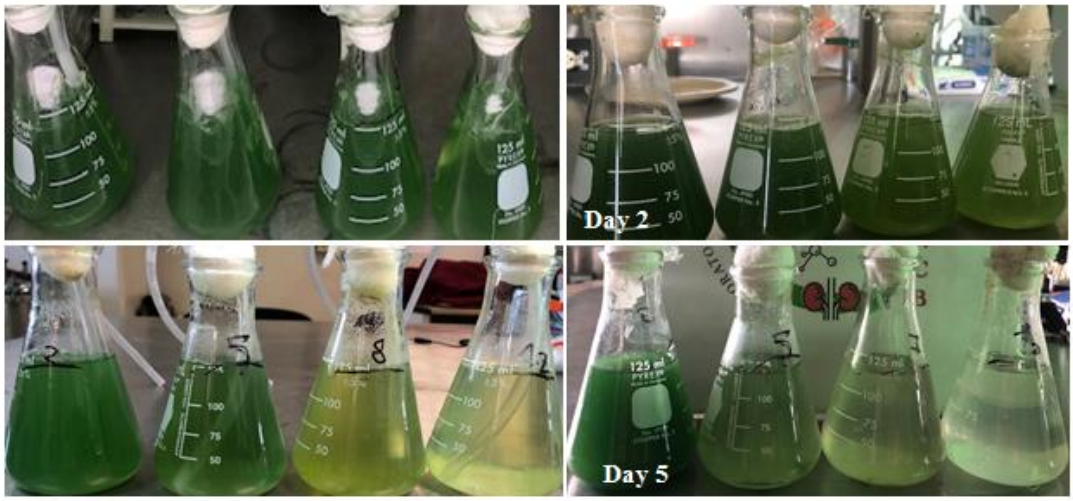

Figure 3: Changes in medium's color during cultivation day (from right to left show four different concentrations of ammonium sulfate of 0, 1.25, 2.5 and 5 gL^{-1}, respectively)

The changes in final cell dry weight and nitrogen-cell conversion factors related to nitrogen sources at different concentrations are presented in Table 2. Variations in the ammonium sulfate concentrations did not show a significant difference in a pattern throughout the growth and the cell dry weight values. The final cell dry weight of treatment 9 was the lowest when ammonium was only twice higher than the control concentration (2.5 gL^{-1}) of the total nitrogen. According to Table 2, the medium without nitrate source seemed more adequate than the others for growth. The nitrogen-cell conversion factor was decreased and cell dry weight decreased, suggesting the negative impact of ammonium sulfate when added to the medium.

The maximum specific growth rate (μ_{max}), doubling time (t_d), and correlation coefficient (r) were studied as a function of nitrogen source according to Table 3.

Table 2: Final cell dry weight and nitrogen-cell conversion factor of *S. maxima* in different treatments.

Treatment No.	1	2	3	4	5	6	7	8	9
Final cell dry weight (gL^{-1})	1.209	1.200	1.010	1.001	0.992	0.835	0.143	0.088	0.065
Nitrogen cell conversion factor (gg^{-1})	ND	0.776	0.306	0.151	0.074	ND	N	N	N

ND: Nitrogen source was not added in the medium
N: Negative amount

S. maxima samples were grown successfully in 5 out of 9 treatments. According to Table 3, the highest maximum specific rate (0.287) was found at 0 gL^{-1} sodium nitrate at a smaller doubling time (2.43). Nevertheless, there were no statistical differences in this group. We recognized that ammonium utilization causes the pH to drop below the normal

[Downloaded from jifro.ir on 2026-11-10]
[DOR: 20.1001.1.15622916.2021.20.2.16.7]

range of microalga growth to sustain further growth (pH≅ 5) in treatment 9. Moreover, pH instability in the presence of ammonium in the medium culture can prevent microalga growth. As a result, the ammonium source shows the lowest performance in biomass growth and phycobiliproteins production.

Table 3: Maximum specific growth rate (μ_{max}), doubling time (t_d) and correlation coefficient (r) at different treatment.

Treatment	μ_{max} (day^{-1})	t_d (day)	r
1	0.287	2.43	0.9943
2	0.260	2.67	0.9968
3	0.259	2.68	0.9980
4	0.256	2.73	0.9955
5	0.255	2.74	0.9968
6	0.248	2.80	0.9965
7	N	N	0.9907
8	N	N	0.9848
9	N	N	0.9893

N: negative amount

Discussion

Nitrogen source is an important issue since *S. maxima* response can vary with different sources of nitrogen (Bezerra *et al*., 2008; Ferreira *et al*., 2010; Avila-Leon *et al*., 2012; Matsudo *et al*., 2012). In this regard, investigating the effect of nitrogen concentration could help to understand the effect of nutrient factor on production of microalga. Microalgae can utilize inorganic nitrogen in any of the three available forms like nitrate, nitrite, and ammonium by reducing the oxidized nitrogen (Cai *et al*., 2013). In the present study, ammonium sulfate and sodium nitrate were used as supplemental nitrogen in the medium.

Cost *et al*. (2001) reported that the highest biomass was obtained in sodium nitrate, ammonium nitrate, and urea, in the order of their appearance (Cost *et al*., 2001). As a result, it was determined that the sodium nitrate at different concentrations introduced into the culture medium had no apparent positive effect on phycocyanin and allophycocyanin content. They also reported that the optimal cell dry weight was 1.992 gL^{-1} in the growth medium containing 0.03 M sodium nitrate for production of *S. platensis* during 672 hours (28 days) cultivation (Cost *et al*., 2001). We have determined that the cell dry weight decreased by increasing the concentration of sodium nitrite from 0 to 10 gL^{-1}, although the sodium nitrate at different concentrations introduced into the culture medium had no apparent positive effect. On the other hand, it was reported that the concentration of sodium nitrate in Zarrouk medium (2.5 gl^{-1}) reduces without losing the productivity as an important cost-saving factor in large-scale mass production of *S. platensis* (Colla *et al*., 2007). Furthermore, de Castro *et al*. (2015) proved that sodium nitrate concentration in the Zarrouk's medium can be reduced by increasing

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

the biomass growth while a higher biomass production depends on the bicarbonate sodium amount (de Castro *et al*., 2015). According to Tedesco and Duerr (1989), total lipid content was increased with nitrogen starvation in the Zarrouk's medium, but the content of fatty acid was decreased in *S. platensis*. Moreover, they reported an increase in biomass growth during the increasing time of nitrogen starvation (Tedesco' and Duerr, 1989). Similarly, in this study, the cell dry weight and pigment content were increased when nitrogen was limited. Furthermore, Abd El-Baky (2003) found that nitrogen concentration reduction in *S. platensis* culture decreases phycocyanin, chlorophyll and protein contents due to break-downing the chloroplasts which was not in accordance with our findings (El-Baky *et al*., 2003). However, nitrogen starvation increased the lipid fraction of some microalgae such as *S. platesis* (Illman *et al*., 2000; Uslu *et al*., 2011; Ak *et al*., 2015). Moreover, a research showed that the highest biomass content of *Spirulina platensis* without nitrogen source during 31 days of cultivation may be obtained while in contrast to the present research, nitrogen content had no significant effect on the biomass production enhancement (Uslu *et al*., 2011). On the basis of *Spirulina platensis*, a sharp decrease on biomass and phycocyanin production was reported when the concentration of sodium nitrate increased to 4.5 gL^{-1}. However, the maximum biomass concentration and phycocyanin productivity were observed in the batch medium containing 3.5 gL^{-1} than the classic amount of Zarrouk's medium in case of *Spirulina platensis* (Kaewdam *et al*., 2019).

Similar to our result, Cost *et al.* (2001) reported that it is impossible to utilize ammonium sulfate as an alternative nitrogen source instead of the classic nitrate-based of Zarrouk's medium for the growth of *S. platensis*. The results showed that biomass growth was decreased by increasing the concentration of ammonium sulfate (Cost *et al*., 2001). Based on sulfate ammonium, it is suggested that the fed-batch supply of ammonium sulfate under pH control and varying the feeding time (time = 7–15 days) on a modified Schlösser's medium leads to the best results in terms of biomass growth, protein, lipid, and carbohydrate content. Moreover, day 13 was selected as the best condition for adding ammonium sulfate on medium (Rodrigues *et al*., 2011).

Moreover, the ammonium sulfate rapidly dissolves into the ammonium and sulfate forms after addition to the culture medium (International Plant Nutrition Institute (IPNI)). Moreover, we also showed that *S. maxima* growth in the culture medium is associated with color variation. Also, the ammonium may be susceptible to gaseous loss in alkaline conditions. Then, ammonium component was converted in to the nitrite by nitrification process ($2NH_4^+ + 3O_2 \rightarrow 2NO_3^- + 2H_2O + 4H^+$) during

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

the cultivation period (Arsalan Sepehri and Sarrafzadeh, 2019). Although ammonia was converted in to the nitrite in the presence of oxygen during the first day of cultivation, converting the produced nitrites into the nitrate form needs more oxygen (Barth *et al*., 2020). Hence, the ammonium sulfate typically has a pH in range of 5 to 6, which is lower than the *pH* balance of the microalga growth. Furthermore, the pH of culture medium was ultimately decreased by volatilization of acidity (H^+). However, this level is not the desired working pH and may lead to death if the microalga is not adequately alkaline (in the range of 8 to 10). The experimental results of this study showed the ability of microalgae death to utilize ammonium and pH drop ($\cong$ 5.3) in medium culture.

Based on the other study, the supply of ammonium as a nitrogen source in the Zarrouk's medium for cultivating *Spirulina platensis* was shown to be effective either to produce biomass or to increase its CPC content. Notwithstanding, the microalgae utilize ammonium on excess CO2 by aeration during the biomass growth to control growth pH at 9.0-9.5 (Kaewdam *et al*., 2019). Moreover, we also showed that *S. maxima* growth in the culture medium related to the observation of changing color from dark green to white during the first five days of cultivation. Besides this, ammonium sulfate did not allow *S. maxima* to settle down and grow, as well as preventing sodium nitrate as a nitrogen source. Therefore, a negative specific growth rate (μ_{max}) and doubling time (t_d) were observed when ammonium sulfate was available in the medium. In this process, using excess bicarbonate with ammonium may be useful while using ammonium can be the principal cause of the reduction in alkalinity, which impacts pH stability and then biomass growth capacity. Another result of this study was that phycocyanin and allophycocyanin contents of S. maxima were rapidly declined by increasing the ammonium sulfate concentration in the medium.

Among the nutrients required for the growth of S. maxima, nitrogen is not considered as an important element for the synthesis of phycobiliprotein according to our findings, while it is almost necessary for the synthesis of proteins and other cellular components (Khazi *et al*., 2018). It has been also reported that nitrogen is essential factor for synthesis of the amino acids and the excess nitrogen can cause an increase in the protein content of Spirulina. The same finding showed that lipid content could remained constant at all concentration of nitrogen (Piorreck *et al*., 1984). With regard to our study, high concentration of nitrate approximately showed no increase of phycobiliprotein content in Spirulina.

Briefly, the use of ammonium sulfate as an alternative source instead of the classic-nitrate source of Zarrouk's medium was not an appropriate strategy for increasing the biomass growth and pigment content of

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

S. maxima. Although the addition of sodium nitrate introduced into the culture medium in high concentration had no positive effect on the biomass growth and pigments production, the absence of a nitrogen source could accelerate the biomass growth and promote pigments production under the conditions tested in this study. Moreover, the biomass growth would be limited by pH drop toward acidity in the medium. Besides, using ammonium sources was also found to be 'toxic' to algae growth. Another result is that sodium nitrate concentration was not sensitive to biomass growth changes of *S. maxima*. According to the experimental results of this study, either sodium nitrate or ammonium sulfate does not affect the final cell dry weight and phycobiliproteins content. This study highlights the advantages of nitrogen starvation both in terms of lower costs and higher biomass growth, which can be used for large-scale production in the real environment.

Acknowledgments

All authors declare there is no conflict of interest for this study. This study was partially supported by SIP-IPN 20200493, 20200521, and 2020109. We thank INSTITUTO POLITÉCNICO NACIONAL, SECRETARÍA DE INVESTIGACIÓN Y POSGRADO-IPN. The Mexicans researchers are fellows of EDI, COFAA, and SNI.

References

Ak, B., Işık, O., Uslu, L. and Azgın, C., 2015. The effect of stress due to nitrogen limitation on lipid content of phaeodactylum tricornutum (Bohlin) cultured outdoor in photobioreactor. *Turkish Journal of Fisheries and Aquatic Sciences*, 15, 647–652. DOI: 10.4194 / 1303-2712-v15_3_09.

Avila-Leon, I., Chuei Matsudo, M., Sato, S. and de Carvalho, J.C.M., 2012. Arthrospira platensis biomass with high protein content cultivated in continuous process using urea as nitrogen source. *Journal of Applied Microbiology*, 112, 1086–1094. DOI: 10.1111/j.1365-2672.2012.05303.

Barth, G., Otto, R., Almeida, R.F., Cardoso, E.J.B.N., Cantarella, H. and Vitti, G.C., 2020. Conversion of ammonium to nitrate and abundance of ammonium-oxidizing-microorganism in tropical soils with nitrification inhibitor. *Scientia Agricola*, 77, 2–6. DOI: 10.1590/1678-992x-2018-0370

Beatriz. M., Gloria. A. and Christian, F., 2015. Handbook of Food Analysis - Two Volume Set - Google Books, in: Leo M.L. Nollet, Fidel Toldra (Eds.), Handbook of Food Analysis. CRC Press, Taylor and France group. London. New York. pp. 105–128.

Bennett, A. and Bogobad, L., 1973. Complementary chromatic adaptation in a filamentous blue-green alga. *Journal of Cell Biology*, 58, 419–435. DOI:10.1083/jcb.58.2.419

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

Bezerra, R.P., Matsudo, M.C., Converti, A., Sato, S. and De Carvalho, J.C.M., 2008. Influence of ammonium chloride feeding time and light intensity on the cultivation of *Spirulina (Arthrospira) platensis*. *Biotechnology and Bioengineering*, 100, 297–305. DOI: 10.1002/bit.21771.

Cai, T., Park, S.Y. and Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*, 19, 360-369. DOI:10.1016/j.rser.2012.11.030.

Carvalho, A.P. and Malcata, F.X., 2003. Kinetic modeling of the autotrophic growth of *Pavlova lutheri*: Study of the combined influence of light and temperature. *Biotechnology Progress*, 19, 1128–1135. DOI:10.1021/bp034083.

Carvalho, J.C.M., Francisco, F.R., Almeida, K.A., Sato, S. and Converti, A., 2004. Cultivation of *Arthrospira (Spirulina) platensis* (Cyanophyceae) by fed-batch addition of ammonium chloride at exponentially increasing feeding rates. *Journal of Phycology*, 40, 589–597. DOI: 10.1111/j.1529-8817.2004.03167.

Clydesdale, F.M., 1993. Color as a factor in food choice. *Critical Reviews in Food Science and Nutrition*, 33, 83–101. DOI: 10.1080/10408399309527614.

Colla, L.M., Oliveira Reinehr, C., Reichert, C. and Costa, J.A.V., 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresource Technology*, 98, 1489–1493. DOI: 10.1016/j.biortech.2005.09.030.

Cost, J.A.V., Cozz, K.L., Oliveira, L. and Magagnin, G., 2001. Different nitrogen sources and growth responses of Spirulina platensis in microenvironments. *World Journal of Microbiology and Biotechnology*, 17, 439–442. DOI: 10.1023/A:1011925022941.

Çelekli, A. and Yavuzatmaca, M., 2009. Predictive modeling of biomass production by *Spirulina platensis* as function of nitrate and NaCl concentrations. *Bioresource Technology*, 100, 1847–1851. DOI: 10.1016 / j.biortech.2008.09.042.

Danesi, E. D., Rangel, Y., Oliveira, C., Sato, S.y Carvalho, J. C. M, 2011. Crecimiento y contenido de clorofila de biomasa de *Spirulina* platensis cultivada a diferentes valores de intensidad de luz y temperatura utilizando diferentes fuentes de nitrógeno . Braz. J. Microbiol, 42, 362-373. DOI: 10.1590/S1517-83822011000100046.

de Castro, G.F.P. da S., Rizzo, R.F., Passos, T.S., dos Santos, B.N.C., Dias, D. da S., Domingues, J.R. and Araújo, K.G. de L., 2015. Biomass production by *Arthrospira platensis* under different culture conditions. *Food Science and*

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

Technology, 35, 18–24. DOI: 10.1590/1678-457X.6421.

Dineshkumar, R., Narendran, R. and Sampathkumar, P., 2016. Cultivation of *Spirulina platensis* in different selective media. *Indian Journal of Geo Marine Sciences*, 45, 1749–1754. DOI: IJMS 45 (12) 1749-1754.pdf.

Ferreira, L.S., Rodrigues, M.S., Converti, A., Sato, S. and Carvalho, J.C.M., 2010. , 26, 1271–1277. DOI: 10.1002/btpr.457.

Illman, A.M., Scragg, A.H. and Shales, S.W., 2000. Increase in *Chlorella strains* calorific values when grown in low nitrogen medium. *Enzyme and Microbial Technology*, 27, 631–635. DOI: 10.1016/S0141-0229(00)00266-0.

IPNI, n.d. Nutrient source SPECIFICS [WWW Document]. International Plant Nutrition Institute . URL http://www.ipni.net/publication/nss.nsf/0/A9E141566F664341852579AF007640CF/%24FILE/NSS-12 Ammonium Sulfate.pdf (accessed 5.15.20).

Kaewdam, S., Jaturonglumlert, S., Varith, J., Nitatwichit, C. and Narkprasom, K., 2019. Kinetic models for phycocyanin production by fed-batch cultivation of the *Spirulina platensis*. *International Journal of GEOMATE*, 17, 187–194. DOI: 10.21660/2019.61.89205.

Kevin, B., Hicks, William F. Fett, Fishman M., Gerald M. Sapers and Arthur M. Spanier, 1992. New Crops, New Uses, New Markets: Industrial and Commercial Products from U.S. Agriculture. Google Books, in: Edward Madgian (Ed.), Natural products to provide flavor, color, and other qualities. pp. 252–255.

Khatun, R., Noor, P., Akhter, N., Jahan, M., Hossain, M. and Munshi, J., 1970. Spirulina culture in Bangladesh XI: Selection of a culture medium, suitable for culturing a local strain of *Spirulina*. *Bangladesh Journal of Scientific and Industrial Research*, 41, 227–234.

Khazi, M.I., Demirel, Z. and Dalay, M.C., 2018. Evaluation of growth and phycobiliprotein composition of cyanobacteria isolates cultivated in different nitrogen sources. *Journal of Applied Phycology*, 30, 1513–1523. DOI: 10.1007/s10811-018-1398-1.

Kuddus, M., Singh, P., Thomas, G. and Al-Hazimi, A., 2013. Recent developments in production and biotechnological applications of C-phycocyanin. *BioMed Research International* , 1–9. DOI: 10.1155/2013/742859.

Kumar, M., Kulshreshtha, J. and Singh, G.P., 2011. Growth and biopigment accumulation of cyanobacterium *Spirulina platensis* at different light intensities and temperature. *Brazilian Journal of Microbiology*, 42, 1128. DOI: 10.1590/S1517-83822011000300034.

Matsudo, M.C., Bezerra, R.P., Sato, S., Converti, A. and De Carvalho, J.C.M., 2012. Photosynthetic

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

efficiency and rate of CO2 assimilation by *Arthrospira (Spirulina) platensis* continuously cultivated in a tubular photobioreactor. *Biotechnology Journal*, 7, 1412–1417. DOI: 10.1002/biot.201200177.

Park, W.S., Kim, H.J., Li, M., Lim, D.H., Kim, J., Kwak, S.S., Kang, C.M., Ferruzzi, M.G. and Ahn, M.J., 2018. Two classes of pigments, carotenoids and c-phycocyanin, in spirulina powder and their antioxidant activities. *Molecules*, 23, 2065. DOI: 10.3390/molecules23082065.

Piorreck, M., Baasch, K.H. and Pohl, P., 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry*, 23, 207–216.

Ashton Acton, Q., 2013. Cyclohexenes—advances in research and application: 2013 Edition: ScholarlyBrief - Google Books, ScholarlyBrief. ed.

Rajasekaran, C., Ajeesh, C.P.M., Balaji, S., Shalini, M., Siva, R., Das, R., Fulzele, D.P. and Kalaivani, T., 2016. Effect of modified zarrouk's medium on growth of different *Spirulina* strains. *Walailak Journal of Sciences and Technology*, 13, 67–75. DOI: 103.58.148.28.

Raoof, B., Kaushik, B.D.D. and Prasanna, R., 2006. Formulation of a low-cost medium for mass production of *Spirulina*. *Biomass and Bioenergy*, 30, 537–542. DOI: 10.1016/j.biombioe.2005.09.006.

Rodrigues, M.S., Ferreira, L.S., Converti, A., Sato, S. and de Carvalho, J.C.M., 2011. Influence of ammonium sulphate feeding time on fed-batch *Arthrospira (Spirulina) platensis* cultivation and biomass composition with and without pH control. *Bioresource Technology*, 102, 6587–6592. DOI: 10.1016/j.biortech.2011.03.088.

Salunke, K. J., Magar S. A., Joshi R. R. and Wadikar, M. S., 2016. Comparative study on growth of Spirulina platensis on different culture media. Bioscience Discovery, 7(**1**), 90-92. DOI: http://jbsd.in.

Sánchez, M., Bernal-Castillo, J., Rozo, C. and Rodríguez, I., 2003. *Spirulina* (*Arthrospira*): an edible microorganism: a review|universitas scientiarum. *Universitas scientiarum revista de la facultad de ciencias*, 8, 7–24.

Sarada, R., Pillai, M.G. and Ravishankar, G.A., 1999. Phycocyanin from *Spirulina* sp: Influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. *Process Biochemistry*, 34, 795–801. DOI: 10.1016/S0032-9592(98)00153-8.

Sepehri, A. and Sarrafzadeh, M.H., 2019. Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]

sludge process: metabolite reduction and CO2 mitigation intensification process. *Applied Water Science*, 9, 1–12. DOI: 10.1007/s13201-019-1017-6.

Sigurdson, G.T., Tang, P. and Giusti, M.M., 2017. Natural colorants: Food colorants from natural sources. *Annual Review of Food Science and Technology*, 8, 261–280. DOI: 10.1146/annurev-food-030216-025923.

Soletto, D., Binaghi, L., Lodi, A., Carvalho, J.C.M. and Converti, A., 2005. Batch and fed-batch cultivations of Spirulina platensis using ammonium sulphate and urea as nitrogen sources. *Aquaculture*, 243, 217–224. DOI: 10.1016/j.aquaculture.2004.10.005.

Tedesco, M.A. and Duerr, E.O., 1989. Light, temperature and nitrogen starvation effects on the total lipid and fatty acid content and composition of *Spirulina platensis* UTEX 1928. *Journal of Applied Phycology*, 1, 201–209. DOI: 10.1007/BF00003646.

Uslu, L., Işik, O., Koç, K. and Göksan, T., 2011. The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*. *African Journal of Biotechnology*, 10, 386–389. DOI: 10.5897/AJB10.1547.

Vo, T.S., Ngo, D.H. and Kim, S.K., 2015. Nutritional and pharmaceutical properties of microalgal *Spirulina*, in: Handbook of marine microalgae: Biotechnology advances. Elsevier Inc., pp. 299–308. DOI: 10.1016/B978-0-12-800776-1.00019-4.

Wissgott, U. and Bortlik, K., 1996. Prospects for new natural food colorants. *Trends in Food Science and Technology*, 7, 298–302. DOI: 10.1016/0924-2244(96)20007-X.

Zarrouk, C., 1966. Contribución á1'étude d'une cyanophycee . Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de Spirulina maxima (Setch et Gardner) Geitl- Tesis. Universidad de París.

[Downloaded from jifro.ir on 2026-11-10]

[DOR: 20.1001.1.15622916.2021.20.2.16.7]