RESEARCH ARTICLE
Bull Biotechnol (2022) 3(1):11-15
https://doi.org/10.51539/biotech.1033326 Jurnal homepage: www.dergipark.org.tr/biotech

Bulletin of Biotechnology

Production of Natural and Functional Pigments in *Arthrospira (Spirulina) platensis* cultivated in Laboratory Conditions

Mesude İsar, Semra Cirik, Gamze Turan*

Ege University, Fisheries Faculty, Aquaculture Department, 35100 Bornova, İzmir, Turkey

*Corresponding author : gamze.turan@ege.edu.tr
Orcid No: https://orcid.org/0000-0002-3610-6347

Received : 07/12/2021
Accepted : 09/04/2022

Abstract: In this study, *Arthrospira (Spirulina) platensis* was cultivated under laboratory conditions at 30 ± 1 °C and 80 µmol.m^{-2}s^{-1} illumination. Intensive cultivation of *Arthrospira (Spirulina) platensis* was carried out in 250 mL, 500 mL and 2 L flasks, and 10 L and 20 L carboys. Zarrouk Medium was used as a nutrient medium. Constant aeration also applied in the 2 L flasks and the carboys. According to the optical density results, continually increase in the biomass yields of *Arthrospira (Spirulina) platensis* observed until the 24th day of the cultivation period. During the experiment, water temperature was recorded as 29.89 ± 0.45 °C, PH 10.88 ± 0.87, oxygen level of 10.18 ± 2.67 mg/L. *Arthrospira (Spirulina) platensis* has a wide range of pigments, including chlorophyll *a*, total carotenoids and phycobiliproteins (protein-rich phycocyanin and phycoerythrin). The primary potential of these pigments seems to be their use as natural dyes, but a growing number of studies have also shown that the functional properties of these pigments related to health benefits and wide pharmaceutical applications. During the cultivation of *Arthrospira (Spirulina) platensis*, chlorophyll *a*, total carotene, phycocyanin and phycoerythrin production were determined daily by using spectrophotometric methods. The study results showed that the amount of chlorophyll *a* increased until the 29th day when the amount of β carotene increased until the 14th day. The highest chlorophyll *a* was 5.46 ± 0.57 mg/gon the 22nd day, the highest total carotene was 1.82 ± 0.25 mg/g on the 7th day. On the 14th day of the experiment, the amount of phycocyanin was 172.85 ± 7.35 mg/g and the amount of phycoerythrin was 75.54 ± 4.98 mg/g.

Keywords: *Arthrospira (Spirulina) platensis* culture; natural and functional pigments; chlorophyll *a*; total carotene; phycocyanin; phycoerythrin

1 Introduction

Arthrospira (Spirulina) platensis is one of the microalgae species cultivated, which is known as the superfood of the 1990s. In addition, it is a prokaryotic algae that has been living in nature for 3.6 billion years. The blue-green algae *Spirulina* contains filamentous microalgae organized microscopic cells. The optimum growth temperature for *Arthrospira (Spirulina) platensis*, which has thermophilic and alkalophilic features, is 35-37ºC. *Spirulina* is an algae species that prefers high PH (9-10) levels and it can be monocultured without contamination problems due to these particularities. The general purpose of its production is to benefit from the richness of its biochemical structure and to provide a protein source to people (Richmond 2000). All photosynthetic organisms, such as *Spirulina*, contain organic pigments to use light energy (Rizzo et al. 2015). There are three main classes of pigments in algea. These pigments are chlorophylls, carotenoids, and phycobilins. Chlorophylls (green pigments) and carotenoids (yellow and orange pigments) are lipophilic and soluble in alcohol, diethyl ether, benzene, and acetone. In addition, phycobilins are hydrophilic and soluble in water (Richmond 2000).

The only chlorophyll they contain is chlorophyll a and its amount is between 0.8-1.5% of their dry weight when the pigment formation of *Spirulina* is scrutinized. Xanthophyll content is quite high in freeze-dried *Spirulina* (6.9 g/kg). Myxoxanthophyll (37%), Beta Carotene (28%), and Zeaxanthin (17%) are the other major inside of carotenoids (Anderson et al. 1991). Consequently, its rich pigment content, *Spirulina* is used as a feed additive for ornamental fish, particularly for goldfish (Miki et al. 1986). *Spirulina* contains C-phycocyanin and allophycocyanin that are light-harvesting pigments in phycobilisomes. Furthermore, these are the pigments in protein structure with the highest economic value found in *Spirulina* (Fox 1996, Leema et al. 2010). Approximately 20% of the protein content of *Spirulina*

consists of phycocyanin, a water-soluble blue pigment. The maximum absorption of phycocyanin is 620 nm (Ciferri, 1983, Cohen, 1997). Vonshak (1997) declared that phycocyanin served as a storage nutrient in *Spirulina*. In addition, it has been reported that phycocyanin, as a natural pigment in the cosmetic, food, and pharmaceutical industries and also can replace synthetic pigments suspected of being carcinogenic (Cohen 1997, Furmaniak et al. 2017). Phycocyanin is an odorless and non-toxic powder and it is commercially known as "Lina blue" (Vonshak 1997).

The other important value of Phycocyanin is its particularity as an antioxidant pigment. And also it is known to reduce microsomal lipid oxidation and to destroy free radicals (hydroxyl and peroxyl). Aforementioned, it has been determined that phycobilin, which is found in phycocyanin, plays a role in the removal of hydroxyl radicals (Vonshak 1997). Phycobilin is utilized as a biochemical isotope in immunoassays, is used in microscopy and cytometry studies, due to its fluorescent properties (Vonshak 1997).

If improper drying methods are used during the processing of *Spirulina*, they cause about 50% of the phycocyanin to be lost. One of the extraction methods, freezing, crushing the cells with a mortar, homogenization (10000 rpm) provided 19.4 ± 0.4 mg of phycocyanin from each 100 mg of dry *Spirulina*. Extraction with water is a very slow method. In experiments with acid, it was observed that phycocyanin deteriorated. Phycocyanin remains intact at 9 ± 1 ºC and in the PH range of 5-7.5. It has been determined that phycocyanin deteriorates at temperatures above 40 ºC (Vonshak 1997).

Chlorophyll, total carotene, and phycobiliprotein (phycocyanin and phycoerithrin) amounts of *Arthrospira (Spirulina) platensis* grown under laboratory conditions were determined in this assessment.

2 Materials and Method

2.1 *Arthrospira (Spirulina) Platensis* and Culture Conditions

1 L glass flasks containing 3 Zarrouk broths were prepared for the production of *Spirulina* under laboratory conditions (Zarrouk 1966). And daylight fluorescent lamps were used to illuminate the cultures. Cultivation experiments were carried out by adjusting the laboratory temperature to 30±1 °C with an air conditioner and a light intensity of 80 µmol m^{-2} s^{-1}. In addition, continuous lighting was applied.

The optical density of *Spirulina* was determined spectrophotometrically by taking the values of the samples taken from *Spirulina* glass flasks into test glass tubes, at a wavelength of 680 nm in a spectrophotometer calibrated with pure water. SP-300 brand spectrophotometer was used for this purpose, the temperature, oxygen, salinity, and PH values of the water were recorded during the experiment, Moreover water temperatures were determined with the help of 0.1 °C sensitive thermometer, and PH values were defined with the help of Orion PH-meter. The amount of dissolved oxygen was measured in the laboratory with the help of an Oxygen-meter.

2.2 Pigment Extraction and Quantification

Chlorophyll-a and total carotene content of *Arthrospira (Spirulina) platensis* were determined by the spectrophotometric method as indicated below. 5 mg of dried *Spirulina* sample was taken and treated with 5 ml of acetone, and the mixture was homogenized for 10 minutes with the help of a homogenizer for this process. It was then extracted in an ultrasound water bath at 70 °C for 10 minutes. In addition, the obtained extract was centrifuged at 3500 rpm for 10 minutes, and the liquid part containing the pigments was taken and read in the Spectrophotometer (SP-300) at 666 nm and 475 nm wavelengths. The values read were placed in the formula below and the amount of chlorophyll-a and total-carotene was determined.

Chlorophyll-a (mg/g) = 13.9 * A666

Total Carotene (mg/g) = 4.5 *A475 (Jensen 1978)

The ordered procedure was followed to determine the Phycocyanin and Phycoerithrin amount of *Spirulina*. The solutions (K_2HPO_4 and KH_2PO_4) to be used for phycocyanin were prepared, before starting the analysis. 5 mg of wet *Spirulina* sample was gathered from the cultured *Spirulina* samples by centrifugation method. K_2HPO_4 and KH_2PO_4 were added in the same proportions. Moreover, the samples were kept in the deep freezer for 10 days and filled and thawed at 12-hour intervals. The samples were kept in the Centrifuge at 3500-5000 rpm for 10 minutes after 10 days and the biliprotein pigments in the liquid part were read at A562, A615, A652 wavelengths in the Spectrophotometer and the amount of phycocyanin and phycoerythrin (mg/L) was calculated using the formulas below (Jensen 1978).

Phycocyanin (mg/L)=(A562)-0.208*(A615)*/5.09

Phycoerythrin (mg/L)=A562-2.41*Phycocyanin amount-0.849/9.62

2.3 Statistical Analysis

During the process, all data of the trials were analyzed using ANOVA in the SPSS program (Özdamar 2009). The whole of data was submitted as mean ± standard deviation.

3 Results

3.1 Biomass Change of *Arthrospira (Spirulina) platensis*

The growth characteristics of *Spirulina* were investigated at a constant light intensity of 80 µmol m^{-2} s^{-1} by keeping the temperature constant at 30 °C in this experiment. It was evaluated that there was an increase in the enema at 80 µmol m^{-2} s^{-1} light intensity until the 24th day when looking at the optical density changes (Figure 3.1). Average water parameters recorded throughout the experiment are presented in Table 3.1.

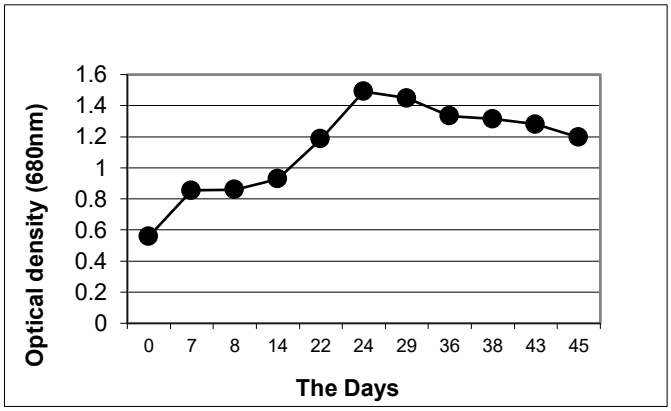


Fig. 3.1 Biomass or optical density of *Arthrospira (Spirulina) platensis* cultivated at 30 °C at 80 µmol m^{-2} s^{-1} light intensity.

Table 3.1 Water quality parameter results in *Arthrospira (Spirulina) platensis* culture units during the experiment

Light intensity (µmol m^{-2} s^{-1})	80
Temperature (°C)	29.89 ±0.45
PH	10.88 ±0.87
Oxygen (mg/L)	10.18 ±2.67

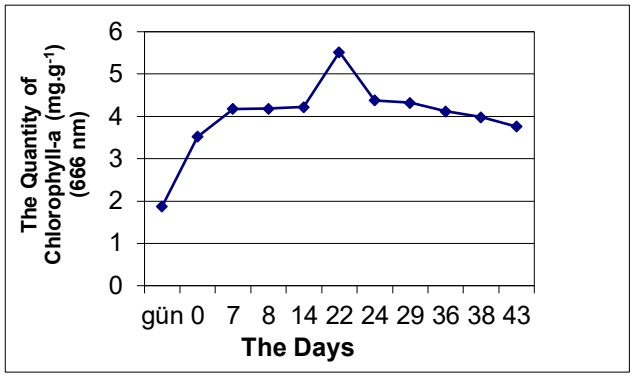
3.2 The Pigment Composition of *Arthrospira (Spirulina) platensis*

It is presented that Chlorophyll-a, Phycocyanin, Phycoerythrin, and total carotene amounts of *Spirulina* clearly in Table 3.2. Following the chlorophyll-a, the amount of *Spirulina* is 5.46 ± 0.57 mg/L on the 22nd day. The phycocyanin amount on the 14th day is 172.85 ± 7.35 mg/L. The amount of phycoerythrin is 75.54 ± 4.98 mg/L and the total carotene amount was found to be 1.82 ± 0.25 mg/g on the 7th day. An increase was observed until the 29th day when the changes in the Chlorophyll-a amount of *Spirulina* were examined (Figure 3.2).

Table 3.2 The average Chlorophyll-a, Phycocyanin, Phycoerythrin, and total carotene of contents of *Arthrospira (Spirulina) platensis*.

Pigments	N=3
Chlorophyll a (mg/g)	5.46 ±0.57
Phycocyanin (mg/L)	172.85 ±7.35
Phycoerythrin (mg/L)	75.54 ± 4.98
Total Carotene (mg/g)	1.82 ± 0.25

Fig. 3.2 Chlorophyll-a content of *Arthrospira (Spirulina) platensis*



cultivated at 30 °C at 80 µmol m^{-2} s^{-1} light intensity.

There was an increase in the total carotene amount until the 14th day when the changes in the total carotene amount were examined (Figure 3.3).

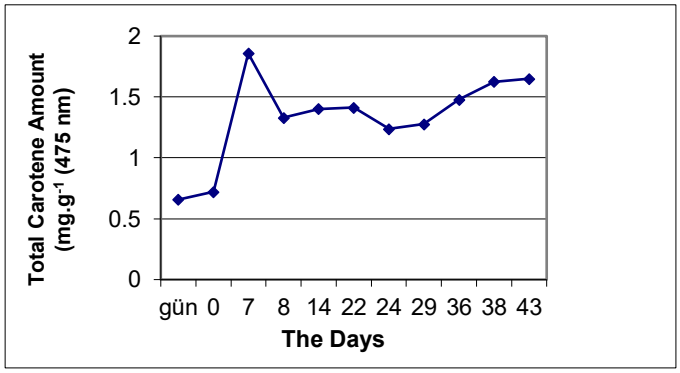


Fig. 3.3 Total carotene content of *Arthrospira (Spirulina) platensis* cultivated at 30 °C at 80 µmol m^{-2} s^{-1} light intensity.

4 Discussion

Cultivation, extraction, and quantification of *Arthrospira (Spirulina) platensis* under laboratory conditions were defined in this study.

Some major conditional processes and steps can be ordered on growth algae. environmental conditions can cause changes in algal growth and algal biochemical structure. It affects growth, nutrient concentrations as well as the type of nutrients used in the nutrient medium (Brown et al. 1989). Hence, Zarrouk nutrient medium was preferred in this study with *Spirulina* (Zarrouk 1966).

Moreover, it is cearly observed that changes in temperature, light and salinity affect the growth and biochemical structures of algae (Vonshak 1997, Rizzo et al. 2015, Kilimtzidi et al. 2019). This examination with *S. platensis* was carried out in Zarrouk nutrient medium, illumination with 60 W fluorescent lamps at 80 µmol m^{-2} s^{-1} light intensity and 30 °C water temperature.

Smayda (1969) defined the photosynthetic rate as the daily carbon assimilation per unit of plant carbon. Baly equation was used as a method model. Briefly, the content of the Baly equation shows that temperature is a variable. It affects the photosynthetic rate. Smayda's work in 1969 showed that light, temperature, and salinity affect the growth of algae. It is known that algal photosynthesis is dependent on light intensity and they are integrated with different photosynthetic pigments. He gave importance to the light needs of different algae species in his studies. In addition, he ascertained that the species in the Chlorophyceae class were saturated with light between 0.032-0.048 ly.minute^{-1}. Emphasizing the importance of pigments at low light intensity. As a result of this, he concluded that chlorophyll and phycocyanin at high light intensity are very important for absorbing light intensity.

There are important points and steps for determining the nutritional quality, nutrient medium, and salt concentration (Kaplan et al. 1986). These are the biochemical composition of algae growth rate and growth phases (Molina-Grima et al. 1996), temperature (Kaplan et al. 1986), lighting (Brown et al. 1993; Molina-Grima et al. 1994), microelements (Akbarnezhad et al, 2020), media and nitrogen resources (El-Sheekh et al. 2021). Carotenes are of vital importance for human health because they protect cell roots and tissues from harmful factors as biological antioxidants. Moreover, many researchers have suggested carotenoids as protective against human diseases, and also carotenes such as β-carotene and lutein have been still used in cancer treatment (Richmond 2000; Ziegler et al. 1996). The main pigments in *Spirulina* are Chlorophyll-a, phycocyanin, phycoerythrin, and beta-carotene. In addition, these algal species contain secondary carotene groups such as canthaxanthin and astaxanthin. Secondary carotenoids are produced under extreme conditions such as high light intensity. Particularly, the change of color from green to red-orange as a result of nutrient restriction has been associated with an increase in secondary carotenes (Lubian et al. 2000). They also reported a decrease in chlorophyll-a and total carotene values due to the increase in light intensity in algae. In this study, the pigment composition and amounts of *Spirulina* were determined with this assessment.

Algal pigments are products presented in the beauty and health products market as various creams, milk, lotions, mud, face, and body masks. Chlorophyll is a green pigment substance that absorbs light of various wavelengths and provides photosynthesis (assimilation) to occur in the plant. Chlorophyll is responsible for absorbing the light energy used in photosynthesis, the reduction of carbon dioxide to other plant substances and sugars. In orderly, all green plants except bacteria contain Chlorophyll a, chlorophyll b is found in higher plants and green algae, red algae includes chlorophyll d, and chlorophyll c is found in diatoms, brown algae, and flagellates such as euglena. It is very important for helping to heal wounds on the skin, treat skin roughness, and accelerate tissue adaptation in skin transplantation. Chlorophyll is used comprehensively as a natural cosmetic product. It has become one of the application areas in cosmetics by being included in olive oil soaps. Furthermore, it is also used in natural cosmetic products such as face care masks and care creams besides soaps. Pigments are a rich source of vitamins, antioxidants, and minerals. These Pigments are algae such as Fucoxanthin, Phycocyanin, Phycoerythrin. It absorbs calories from the foods that people consume and prevents them from turning into fat. It helps for using in the treatment of cellulite, and also it has the impact of strengthening the immune system. Carotene has a significant role in photosynthesis as a photosynthetic pigment. Carotene can be stored in the liver and converted to vitamin A when needed, consequently, it's considered a provitamin. It s to photosynthesis by transferring the absorbed light to chlorophyll. It is responsible for providing the orange color of carrots and most other vegetables and fruits. β-Carotene is the widespread form. Leafy fruits and vegetables such as colors green, orange or yellow are contained a high level of β- carotene. In addition, brown algae contain carotene. Carotene is also effective against skin aging besides protecting the skin from the harmful rays of the sun (Cirik 1989, Cirik and Cirik 1999, Cirik and Gökpınar 1999; Cirik and Conk-Dalay, 2001;Koru and Cirik 2003).

5 Conclusion

In the scope of this research, aquaculture protocols and pigment compositions of *Arthrospira (Spirulina) platensis* species, which are naturally distributed in our waters, were examined in laboratory conditions. Their potential usage particularly in the field of cosmetics, neutroceuticals, and food should be studied in future research activities.

Acknowledgements

Authors thank to Ege University Scientific Research Projects Programme (BAP- Project number-12-SÜF-022) for providing funding for this study which is a part of thesis study of Bachelor of Science through a grant for Mesude İSAR to pursue her Bachelor of Science Degree at Ege University, Fisheries Faculty, Aquaculture Department, İzmir, Turkey.

Conflict of interest disclosure:

No conflict of interest was declared by the authors.

References

Akbarnezhad M, Shamsaie Mehrgan M, Kamali A, Javaheri Baboli M (2020) Effects of microelements (Fe, Cu, Zn) on growth and pigment contents of Arthrospira (Spirulina) platensis. Iran. J Fish Sci 19(2):653-668

Anderson DW, Tang CS, Ross E (1991) The Xantophylls of *Spirulina* and Their Effect. Poult Sci J 70:115 p

Brown MR, Jeffrey SW, Garland CD (1989) Nutritional aspects of microalgae used in mariculture: a literature review. CSIRO Mar Lab Rep 205:44

Chen F, Zhang Y (1997) High Cell Density Mixotrophic Culture of *Spirulina platensis* on Glucose for Phycocyanin Production Using a Fed-Batch System.Elsevier Science Inc. Enzyme and Microbial Technology, 20:221-224

Ciferri O (1983) *Spirulina*, The Edible Microorganism, Microbiol Rev 47:551.

Cirik S (1989) Zengin Bir Bitkisel Gıda *Spirulina*. Tübitak Bilim ve Teknik Dergisi, Sayı: Nisan, s. 9-20.

Cirik Ş, Cirik S (1999) Su Bitkileri (Deniz Bitkilerinin Biyolojisi, Ekolojisi, Yetiştirme Teknikleri). E.Ü. Su Ürünleri Yayınları, No: 58, s.135-155, Bornova-İzmir

Cirik S, Conk-Dalay M (2001) *Spirulina platensis'* in Açık Hava ve Sera Koşullarında Üretimi. Su Ürünleri Dergisi Cilt No:18/1, s. 117-127, Bornova-İzmir

Cirik S, Gökpınar Ş (1999) Plankton Bilgisi ve Kültürü. E. Ü. Su Ürünleri Fak. Yayınları, No:47, Ders Kitabı 2. Baskı, s. 274, Bornova-İzmir

Cohen Z (1997) The Chemicals of *Spirulina*, 175-204, *Spirulina platensis* (Arthrospira): Physiology, Cell Biology and Biotechnology, Vonshak A (Ed.) Taylor and Francis, London, 226 p.

El-Sheekh MM, Hassan LHS, Morsi HH (2021) Growth Enhancement of Spirulina platensis through Optimization of Media and Nitrogen Sources. Egypt. J Bot 61:61-69

Fox RD (1983) *Spirulina* Production & Potential. Sarl Edisud, La Calade, Rn 7, 3120, 232 p, France.

Furmaniak, MA, Misztak AE, Franczuk MD, Wilmotte A, Walero M, Waleron KF (2017) Edible Cyanobacterial Genus *Arthrospira*: Actual State of the Art in Cultivation Methods, Genetics, and Application in Medicine. Front Microbiol 8:2541

Jensen A **(**1978) Chlorophylls and Carotenoids. In: Handbook of Phycological Methods, Eds: Hellebust, J.A., and Craigie, J.S. Cambridge University Press, pp: 59-70.

Kaplan D, Richmond A, Dubunsky Z, Aaronson S (1986) Algal nutrition. In: Richmond A (Ed), Handbook of Microalgal Mass Culture. CRC Press, FL, pp:147-198

Kilimtzidi E, Bermudez SC, Markou G, Goiris K, Vandammed D, Muylaerta K (2019) Enhanced phycocyanin and protein content of Arthrospira by applying neutral density and red light shading filters: a small-scale pilot experiment. J Chem Technol Biotechnol 94:2047–2054

Koru E, Cirik S (2003) *Spirulina platensis* (Cyanophycea) Mikroalg'inin Büyümesine Ve Bazı Biyokimyasal Özelliklerine Sıcaklığın Etkisi. E.U. J Fish Aquat Sci (3-4):419-422

Leema JTM, Kirubagaran R, Vinithkumar NV, Dheenan PS, Karthikayulu S (2010) High value pigment production from Arthrospira (Spirulina) platensis cultured in seawater. Bioresour Technol 101:9221-9227

Lubian LM, Montero O, Moreno-Garrido I, Huertas IE, Sobrino C, Gonzales-Del Vale M, Pares G (2000) *Nannochloropsis* (Eustigmatophyceae) as source of commercially valuable pigments. J Appl Phycol 12:249-255

Miki W, Yamaguchi S, Konosu S (1986) Carotenoid Composition of *Spirulina maxima*, Bull Jpn Soc Sci Fish 7:1225

Molina-Grima E, Fernadez-Sevilla JM, Sanchez-Perz JA, Garcia Camacho F (1996) A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking in to account incident and averaged irradiances. J Biotechnol 45:59-69

Özdamar K (2009) Paket programlar ve İstatistiksel veri analizleri 1. Kaan Kitapevi, Eskişehir.

Richmond A (2000) Microalgal biotechnology at the turn of the millennium: A personal view. J Appl Phyco 12:441-451

Rizzo RF, Santos BNC, Castro, GFPS, Passos TS, Nascimento MA, Guerra HD, Silva CG, Dias DS, Domingues JR, Lima-Arauj KG (2015) Production of phycobiliproteins by Arthrospira platensis under different lightconditions for application in food products. Food Science and Technology, Campinas, 35(2): 247-252, Abr.-Jun. 2015 247

Smayda TJ (1957) Limnol. Oceanogr. 2:342-359, 1969. J. Phycol. S.150-157, 1970. Oceanogr Mar Biol Annu Rev 8:353-414

Vonshak A (1997) *Spirulina platensis* (*Arthrospira*): Physiology, Cell biology and Biotechnology. Taylor and Francis, London, Great Britain, pp, 213-226

Zarrouk C (1966) Contribution à l'étuded'unecyanophycée. Influence de Divers Facteurs Physiques et Chimiques Sur la Croissance et la Photosynthèse de Spirulina maxima. Retrieved December 18, 2020, from https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1332043

Ziegler RG, Cloavio EA, Hartge P, McAdams MJ, Schenberg JB, Mason TJ, Fraumeni JFJ (1996) Importance of a-carotene, b-carotene and other phytochemicals in the etiology of lung cancer. J Natl Cancer Inst 88:612-615