www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.5, pp 2971-2978, Aug-Sept 2014

Assessment of growth, production and nutritional composition of *Spirulina platensis* under induced conditions of Light regime, Urobilin Addition and H_2O_2 stress

Noorun Nazaha Rasheed[1]*, K.Dhevandran[2]

School of chemical engineering and Biotechnology, SASTRA University, Thirumalaisamudram, Thanjavur, India

***Corres.author: noorunrasheed@gmail.com**

Abstract: *Spirulina platensis* is a blue green algae, which holds marvelous nutritional value and commercial importance. Cultivation of *Spirulina platensis* is investigated under different conditions supplementing with inducers and the criteria such as specific growth rate, biomass accumulation, chlorophyll content, phycocyanin percentage, protein concentration and anti-oxidant potential were analyzed and quantified. The strain of algae *Spirulina platensis*, which was a gift from Bioelixir pvt ltd, Thanjavur was cultivated in Zarrouk's medium. Different light regime were optimized for the growth. The study carried out revealed that higher the light intensity, greater the enhancement in growth. The additional light is preferable in terms of specific growth rate (0. 303day-1), protein concentration (62%), biomass accumulation (0.88g/l), chlorophyll content (3.5 mg/g), phycocyanin content (10.1%) and its respective antioxidant potential (63%). Inducers of phycocyanin production using Urobilin and H_2O_2 were studied where H_2O_2 established its efficiency in promoting phycocyanin content (10.5%) and respective anti oxidative potential (68.7%). Hence Light and H_2O_2 serves as excellent factors for yield improvement in *Spirulina platensis* and greater expression of phycocyanin content which is of pharmacological importance, is resulted. Through this study, the ways to cultivate and harvest *Spirulina platensis* without the loss of its inbound nutrients is investigated and established.
Keywords: *Spirulina platensis*, Light, Urobilin, H_2O_2, Phycocyanin, Antioxidant Potential.

Introduction

Micro algae are photosynthetic green algae or blue-green algae microorganisms that are found in marine and fresh water which utilize solar/light energy, carbon dioxide, and minerals within the water to grow, for the production of high-value compounds and various products.[1][2]

Spirulina is a cyanobacterium,a blue green algae, that can be consumed by humans and other animals and is made primarily from two species of cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima.* It is one of the primitive forms of life on the planet which is commercially produced and widely marketed as super food and an immune booster.[1]

Spirulina platensis are free-floating filamentous cyanobacteria characterized by cylindrical, multi cellular trichome in an open left-hand helix. They occur naturally in tropical and subtropical lakes with alkaline pH and greater concentrations of carbonate and bicarbonate. The largest commercial producers of *Spirulina* are located in the United States, Thailand, India, Taiwan, China, Bangladesh, Pakistan, Myanmar, Greece and Chile.[3]

Dried *Spirulina platensis* contains about 51–71% protein, Lipid 5-7%, Fiber 8-10%, Chlorophyll 1 - 3.5%, Total Carotenoid 0.2-0.4%, Phycocyanin 2.5-9.5% and trace elements. The growth kinetics and composition of *Spirulina platensis* completely rely on the cultivation conditions[6]. Algae are cultivated in one of three ways: photoautotrophically, mixotrophically or heterotrophically.[3][4][5]

The types of pigments in *Spirulina platensis* includes phycoerythrin (red pigment) and phycocyanin (blue pigment).Phycocyanin, a blue natural pigment, is of high importance since it have the potential properties of anti-aging, antioxidant, and anti-inflammatory activities and also effective to defeat cancer metastases.[3][8]

Urobilin, also called as urochrome ,is a yellow compound formed due to degradation of the cyclic tetrapyrrole heme, and is ultimate cause for the yellow color of urine. It has ability in supporting the growth of microalgae better than the basal medium.[7]

Hydrogen peroxide is a byproduct of microalgae produced through photoreactions, and metabolic processes in association with enzyme interaction. H_2O_2 stress possibly has influential role acting as a constructive factor for anti oxidative substances production in *Spirulina platensis* for commercial and pharmaceutical purpose.[2][5][9]

Materials and Methods

Sample Collection

The Sample strain was collected from Bio elixir, nellupattu-pudur post, Thanjavur where *Spirulina platensis* cultivation is done and marketed commercially.

Cultivation

Spirulina platensis was grown in Zarrouk's medium with the initial medium pH 8.5 holding the following composition :$NaHCO_3$, 16.601(g/l); K_2HPO_4, 0.647(g/l); $NaNO_3$, 2.470(g/l); NaCl,0.988(g/l); $MgSO_4$, 0.096(g/l); K_2SO_4, 0.988(g/l); $CaCl_2$, 0.040(g/l); $FeSO_4$, 0.005(g/l);EDTA–Na_2, 0.080(g/l);. Cultures were incubated at room temperature of 30 ± 2°C and during the process of growth the flasks were shaken 3 to 4 times per day for proper mixing of contents.[10][11]

Inducer Studies

It has been with reference to reviews and articles that some factors have considerable impact over the growth of *Spirulina platensis* and its nutritional content increment. To establish the relationship between inducers and *Spirulina platensis* growth, three inducers such as light, urobilin and H_2O_2 were studied and the following parameters such as Growth rate, chlorophyll content, protein content, Phycocyanin content and its respective anti-oxidant potential were analyzed with regard to inducer's impact.[12]

Effect of light

The growth of *Spirulina platensis* was studied under two light regimes. One in presence of normal sunlight and other in addition to the inclined sunlight also illumination was provided over the tank. The white fluorescent lamp was fixed over the tank for the even distribution of photons to the cells. The culturing was carried out at 12 hours light and 12 hours darkness. The growth is analyzed and compounds were quantified.[11][13]

Effect of urobilin

Urobilin is a compound that is present in urine which is excreted by kidney after breaking up the compound urobilinogen. It can act as a stimulator for the growth of *Spirulina platensi*. So it was added at different concentration (2%, 5%, 10%, 15%, 20% & 25%) to the Zarrouk's medium and is inoculated with the *Spirulina platensis*. The respective growth was monitored and Contents were tested.[7][18]

Effect of H_2O_2

Spirulina platensis was cultured in flasks containing Zarrouk's medium, enriched with H_2O_2 (30%, w/v) at stock in concentrations of 0, 2, 4, 6 and 8 mM. The pH of the medium was adjusted with 1 M NaOH

prior to inoculation and cultivated at room temperature. The cultivated flasks were illuminated 12h with continuous cool white fluorescent lamps.

Analytical methods

Growth Kinetics of The Strain

Biomass concentration (gl^{-1}) was calculated by measuring dry weight where biomass were filtered through cheese cloth and oven dried at 75°C for 4 to 6 hours.[19]

Growth kinetics is calculated using the following equation

Specific growth rate(%) = $(\ln W_2 - \ln W_1) / (T_2-T_1)$

Where W_2= Dry weight (g) at T_2, W_1 =Dry weight (g) at T_1; T=Time in days.

Doubling time (hours) =$[\ln (2) / \log[(w_2)/(w_1)]] \times (T_2-T_1)$

Where W_2= Dry weight (g) at T_2, W_1 =Dry weight (g) at T_1; T=Time in days.

Quantitative analysis of Phycocyanin

30 mg *Spirulina* powder was weighed into a 10-ml centrifuge tube and 10ml of the 100mM phosphate buffer (1 00-mM Phosphate buffer contains 10.64g. K_2HP0_4 and 5.298.KH_2PO_4 per liter, pH 7) was added. It was vortexed to mix well and Stored in refrigerator overnight. It was centrifuged for 5 minutes at 3500 rpm. Absorbency at 620 nm**,** using phosphate buffer as blank was read.[12][17]

Derivation of Crude C- Phycocyanin

% Crude CPC = A_{620} x 10 x 100 / (3.39 x mg sample x % dry weight)

Where, 3.39-extinction of co efficient of CPC at 620 nm

10 - Total volume

Protein Estimation

The protein content of the strain was analyzed by means of Lowry method. Standard Lowry chart was made with Bovine serum Albumin. The absorbance is read at 560 nm.[15]

Chlorophyll Content Estimation

Chlorophyll A Assay

50 mg of *Spirulina* weighed approximately into a 35ml centrifuge tube and 5 grams of glass beads and 2.5 ml of 85 % acetone in water were added and Vortexed vigorously for 5 minutes. 10 ml of 85% acetone was added, vortexed and centrifuged at 3200 rpm for 20 minutes. The supernatant was collected in flask and made upto 50 ml .Steps were repeated until supernatant is clear. Four extractions should be sufficient. The absorbency with the spectrophotometer at 666nm and 642 nm was read against an 85 % Acetone/water blank.[14][16]

Chlorophyll a % calculation

Chlorophyll a % = $[\{(9.93 \times Abs_{666})-(0.0777 \times Abs_{642})\} \times 0.05\,l \times 100] / [$ sample weight (mg) x % dy weight]

Determination of Anti oxidant activity

Anti oxidant activity(free radical scavenging activity) was measured using 1, 1-diphenyl-2-picrylhydrazyl (DPPH). 2ml of 0.1 mM DPPH solution in ethanol was added to 2 mL of sample solution (25 mg of dry sample in 100 ml of distilled water). The reaction mixture was stored in darkness for 30 min and the absorbance was measured at 517 nm. Lesser the absorbance, higher the free radical scavenging activity. The ability to scavenge the DPPH radical was calculated using the following equation.

Anti Oxidant activity % = $[(A_0-A_1)/A_0] \times 100$

Where A_0 is the absorbance of control and A_1 is absorbance of the sample.

Results and Discussions

The *Spirulina platensis* was analyzed for its composition and it was observed under microscopy for its structure. The species seems to be possessed of increased phycocyanin content. As per the statement of Bogahawatte *et al*., 2013,*Spiulina platensis* possessed large nutritional emphasis. Growth of *Spirulina platentis* was analyzed day by day. The Organism started growing at faster rate after sixth day of inoculation. The pH of the medium increased as the culture started growing and reached maximum at 11.7.*Refer* Table 1,Fig 1, Fig 2, Fig 3.

Table 1: Growth of *Spirulina platentis*

Day	OD @ 600 nm	pH
1	0.081	8.5
2	0.101	8.6
3	0.152	8.8
4	0.217	9.0
5	0.272	9.2
6	0.333	9.3
7	0.386	9.6
8	0.465	9.9
9	0.523	10.1
10	0.606	10.5
11	0.697	10.8
12	0.778	11.2
13	0.893	11.4
14	1.021	11.7

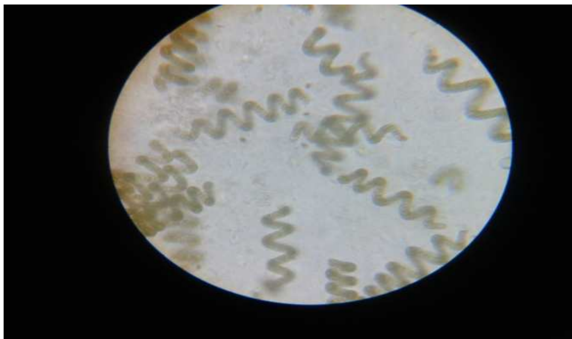

Fig 1: Microscopic structure of *Spirulina platensis*

Fig 2: Cultivated *Spirulina platentis*

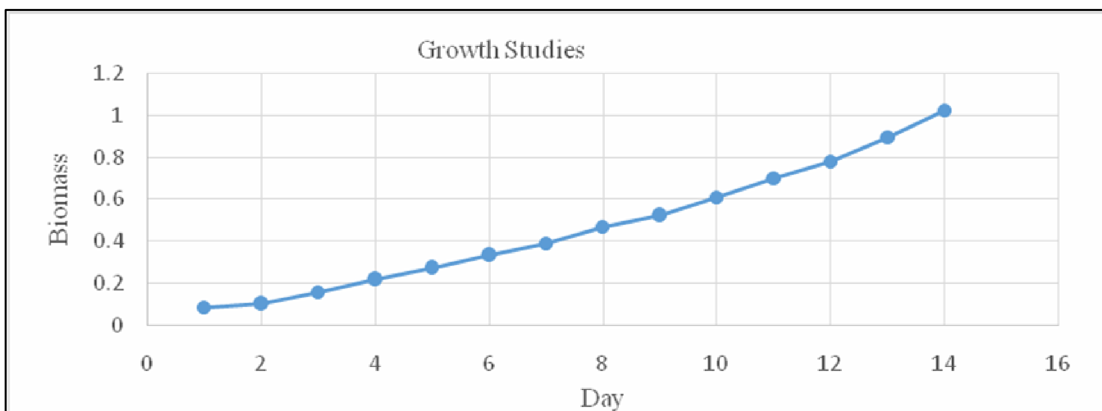

Fig 3: Growth Curve study of *Spirulina platensis*

Impact of light as inducer

The *Spirulina platensis* culture aided with light yielded higher nutritional contents and supported rapid growth rate at short span of time[20][21]. In the study, it had been found that the light had an significant role in the culture growth, the productivity and in influencing the Compounds synthesis. The same can be proved for the blue pigment, Phycocyanin as per the statement of Sandra Lopes Moreira, 2009. *Refer* Table 2, Table 3,Fig 4, Fig 5.

Table 2: Impact of light on Growth of *Spirulina platensis*

Day	Absence of Light	Presence of light
1	0.081	0.031
2	0.101	0.052
3	0.152	0.101
4	0.217	0.161
5	0.272	0.234
6	0.333	0.271
7	0.386	0.397
8	0.465	0.535
9	0.523	0.684
10	0.606	0.929
11	0.697	1.535
12	0.778	2.025
13	0.893	
14	1.021	

Table 3: Growth Kinetics and nutritional content under light regime

Quantification	In absence of Light	In presence of light
Dry weight g/l	0.43	0.88
Growth rate/day	0.181	0.303
Chlorophyll Content mg/g	1.1	3.5
Protein Content%	58	62
Phycocyanin Content%	8.4	10.1
Antioxidant potential %	40.3	63

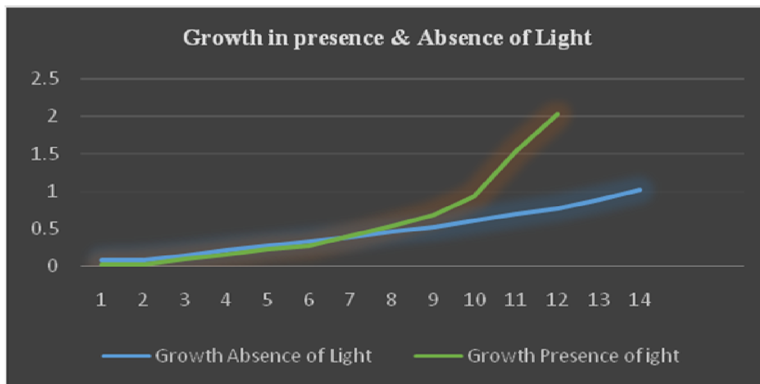

Fig 4: Growth curve studies with reference to light

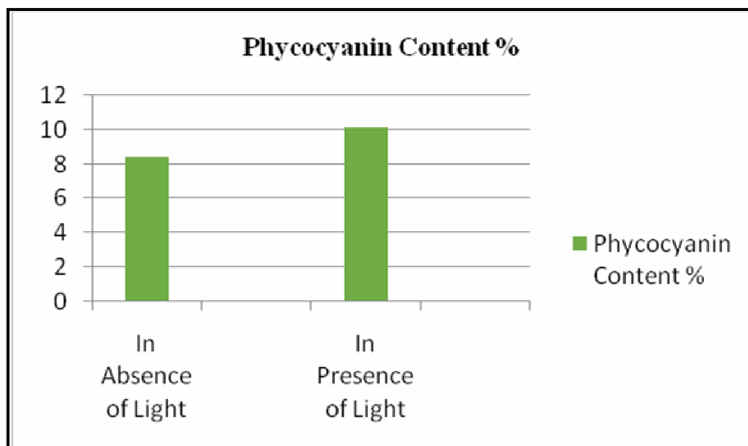

Fig 5: Phycocyanin Content under diff light regime

Urobilin Studies for *Spirulina platensis*

Spirulina platensis was grown at faster rate initially on urobilin addition and later growth declined and compared to control, the growth is not improved considerably. Feng *et al*.,2006 showed that urine can serve as alternate source of medium in space missions. That concept contradicted in our study. *Refer* Table 4.

Effect of H_2O_2

H_2O_2 addition on *Spirulina platensis* growth was checked and analyzed for its impact on improved phycocyanin content.10mM H_2O_2 possessed notable effect on *Spirulina plantensis*'s nutritional value. Similarly, Baky *et al*.,2007 established that H_2O_2 has pronounceable effect on anti oxidant compound production though it won't improve biomass concentration. *Refer* Table 5, Fig 6.

Table 4:Effect of urobilin on *Spirulina platensis* growth

Day	NoUrobilin	5% Urobilin	10% Urobilin	15% Urobilin	20% Urobilin	25% Urobilin
1	0.01	0.01	0.01	0.01	0.01	0.01
2	0.024	0.026	0.013	0.017	0.019	0.023
3	0.049	0.038	0.024	0.039	0.041	0.057
4	0.087	0.093	0.051	0.081	0.086	0.097
5	0.156	0.146	0.135	0.165	0.177	0.178
Urobilin added	0.21	0.217	0.201	0.232	0.251	0.255
6	0.271	0.311	0.297	0.305	0.315	0.327
7	0.346	0.421	0.432	0.315	0.397	0.432
8	0.482	0.333	0.596	0.547	0.571	0.632
9	0.611	0.331	0.667	0.817	0.411	0.667
10	0.789	0.309	0.436	0.653	0.282	0.631
11	0.881	0.291	0.411	0.537	0.256	0.598
12	1.206	0.28	0.321	0.211	0.211	0.552

Table 5: Impact of H_2O_2 on phycocyanin content

H_2O_2 concentration	Phycocyanin Content %	Antioxidant Potential %
0	9.1	40.1
1	8.5	42.3
3	8.2	38.5
5	8.9	42.6
7	9.2	51.3
10	10.5	68.7

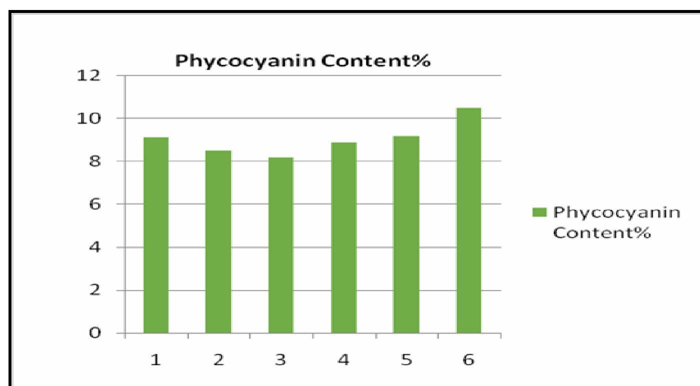

Fig 6: H_2O_2 impact on phycocyanin content

Conclusion

Spirulina platensis was efficiently cultivated and harvested in Zarrouk's medium. The nutritional Analysis of *Spirulina platensis* was done initially to establish its significant role in nutraceuticals and its need for commercialization. Inducer studies were done with respect to light inclination, Urobilin and H_2O_2 to check any possibility of improvement in yield of *Spirulina platensis*. The corresponding factors for phycocyanin content increase and its associated antioxidant potential were studied. The study focused its concern on propagating *Spirulina platensis* as a efficient source of nutrition with induced antioxidant property and revealed that additional light and H_2O_2 promotes the nutrient composition of *Spirulina platensis.*

References

1. C. Bogahawatte, Production and application of Spirulina platensis rich in fatty acids, and vitamins, International Journal of Agr. & Env. [01] (2013).
2. Hanaa H. Abd El-Baky, F.K. El Baz and Gamal S. El-Baroty, Enhancement of Antioxidant Production in *Spirulina Plantensis* Under Oxidative Stress, American-Eurasian Journal of Scientific Research 2 (2) (2009) 170-179.
3. Sandra Lopes Moreira, Reactor design for a family production of Spirulina spp. and parameters determination for a *Spirulina spp*. culture, Master Thesis Development Project in Foreign Institution, 2009.
4. Shengzhang Xuea, Zhenfeng Sua, Wei Conga, Growth of *Spirulina platensis* enhanced under intermittent illumination, Journal of Biotechnology 151 (2011) 271–277.
5. Hua-Bing Chena, Jiun-Yan Wua, Chin-Feng Wanga, Chun-Chong Fua, Chwen-Jen Shiehb, Chih-I Chenc , Chih-Yu Wanga, Yung-Chuan Liua, Modeling on chlorophyll a and phycocyanin production by *Spirulina platensis* under various light-emitting diodes, Biochemical Engineering Journal 53 (2010) 52–56.
6. H.K. Madhyastha, T.M. Vatsala, Pigment production in *Spirulina fussiformis* in different photophysical conditions, Biomolecular Engineering 24 (2007) 301–305.
7. Dao-lun Feng and Zu-cheng Wu , Culture of *Spirulina platensis* in human urine for biomass production and O_2 evolution, J Zhejiang journal of science letters,7(1), PMCID: PMC1361757B, (2006) 34–37.
8. Pinero Estrada, J. E, Bermejo Bescos, P.,Villar Del Fresno, A. M. (2001). Antioxidant activity of different fractions of Spirulina platensis protean extract,*Farmaco (Societa chimica italiana)* 56 (5–7) (1989) 497–500.
9. Ratana Chaiklahan , Nattayaporn Chirasuwan, Veara Loha, Suvit Tia, Boosya Bunnag, Separation and purification of phycocyanin from *Spirulina sp*. using a membrane process, Bioresource Technology 102 (2011) 7159–7164.
10. Jai Prakash Pandey, Amit Tiwari, Optimization of Biomass Production of *Spirulina maxima*, J. Algal Biomass Utln.**1** (2) (2010) 20-32.
11. Blanken , Maria Cuaresma , René H.Wijffels , Marcel Janssen, Cultivation of microalgae on artificial light comes at a cost, Algal Research 2 (2013) 333–340.
12. McCarty, M. F., Clinical Potential of *Spirulina* as a Source of Phycocyanobilin. *Journal of Medicinal Food* 10 (4) (2007) 566–570.
13. Ogbonna, J.C., Soejima,T., Tanaka, H., An integrated solar and artificial light system for internal illumination of photobioreactors. J.Biotechnol. 70, (1999) 289–297.
14. Reinehr, C.O., Costa, J.A.V., Repeated batch cultivation of the microalga *Spirulina platensis*. World J. Microb. Biot. 22, (2006) 937–943.
15. Cynthia Victoria Gonzalez Lopez, María del Carmen Ceron García, Francisco Gabriel Acién Fernandez ,Cristina Segovia Bustos, Yusuf Chisti, José Maria Fernández Sevilla, Protein measurements of microalgal and cyanobacterial biomass, Bioresource Technology 101 (2010) 7587–7591.
16. E. Molina Grima, F.G. Acien Fernandez, F. Garcıa Camacho, Yusuf Chisti, Photobioreactors: light regime, mass transfer, and scaleup, Journal of Biotechnology 70 (1999) 231–247.
17. J.T. Mary Leema , R. Kirubagaran, N.V. Vinithkumar , P.S. Dheenan , S. Karthikayulu, High value pigment production from Arthrospira (Spirulina) platensis cultured in seawater, Bioresource Technology 101 (2010) 9221–9227.
18. Chang, Yuanyuan., Cultivation of *Spirulina platensis* for biomass production and nutrient removal from synthetic human urine, Applied Energy 102 (2013) C 427-431.

19. S.K.Soni, Kamal Agrawal, S.K. Srivastava, S. Gupta, and Chetan Kumar Pankaj.,Growth performance and biochemical analysis of *Spirulina platensis* under different culture conditions, *J*. Algal Biomass Utln. (2012) 3 (1) ,55- 58.
20. Y.K. Lee., Enclosed bioreactor for the mass cultivation of photosynthetic microorganisms: the future trend. Trends Biotechnol. 4, (1986) 186–189.
21. Fernandez, Camacho F., Sanchez, Perez.. A model for light distribution and average solar irradiance inside outdoor tubular photobioreactors for microalgal mass culture. *Biotechnology Bioengineering* **55**, (1997) 701-714.

*****