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PRODUCTIVITY OF *Spirulina fusiformis,* (VORINICHIN) IN PLASTIC PHOTOBIOREACTOR WITH SUN LIGHT FILTERING

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ABSTRACT

Recently climate change has become a complex issues which have relation with many problems in the world, one of them is food security. Based on these problems, we need alternative food resources which have high productivity characteristic. Besides that, such resource should be able to be grown in critical and limited land. Microalgae as an alternative food resource give us a hope to have sustainable food resource in the future. Microalgae can grow up fast and do not need wide and fertile land. In other hand, Microalgae culture that can be performed in outdoor, especially Spirulina fusiformis, faced issues like expensive cost culture and the high intensity of sun light in field. Based on these problems, the research was conducted for develop cheap flat Photobioreactor (plastic) which is equipped by sun light filter system. The objective of this research is to find out the percentage of sun light filtering that can give high productivity and high protein content. The method used was experimental method, using a Completely Randomized Design (CRD) with six treatments (percentage of the filtering by sunlight 0%, 50%, 60%, 70%, 80%, and 90%.) and four replications.. Parameters measured were growth, levels of protein, pH, temperature, and light intensity. Research results showed filter sun light treatment influential real against productivity S. fusiformis. The highest productivity is obtained at 50% filter sun light tretament (F5), in the amount of 0.063 g/L/day. The highest protein content obtained at 70% sun light filtering treatment, which is equal to 49.83 %.

Keywords: Flat Photobioreactor, Plastic Photobioreactor, Productivity, Photo inhibition, Spirulina fusiformis, Sunlight Filtering.

INTRODUCTION

Recently climate change has become a complex issue which has relation with many problems in the world; one of them is food security. FAO data show that the number of people under starving threat in the world is estimated to reach 925 million people by 2010 and in Indonesia amounted to 29.9 million people in the period 2005-2007 (FAO, 201 I). Every year, starving in the world is increasing 5.4 million people and died reached 36 million people (Rosario, 2007).

The problem of hunger is not only caused by economic factors, but by many factors such as discharge of field, land use change, critical land and climate change (FAO, 2011). Therefore, the handling is not enough through economic approach. It is also required the development of alternative food sources which have characteristic such as high nutrition, high productivity, high efficiency use of land and high endurance from climate change. One of the sources of food that has these characteristics is spirulina.

Spirulina fusiformis is a microalgae that has protein content up to 50-60% of the dry weight, so it is potential to be developed as a source of protein (Chrismadha, 2006). Microalgae have high productivity and can be developed on critical lands (Ahsan, 2008). In one hectare, *S. fusiform* is able to produce as much biomass as 18-29 tons/ha/year (Chrismadha, 2009). Assuming a 60% protein content, spirulina is able to produce proteins up to 20 times higher than soybeans and 200 times higher than beef in the same area (Ahsan et al, 2008 & Belay, 2005).

Development of microalgae is still constrained by low mastery of culture techniques to produce high productivity with low cost. Various efforts are done to improve the productivity and lower the cost of culture, through optimization of growth parameters and development of simple photobioreactor made from economical material (Chrismadha, 2009).

Photobioreactor is a closed system of microalgae culture techniques that can improve the productivity of microalgae 2-5 times higher than normal conditions (Barsanti Setiawan et al, 2006 & 2008). Culture were performed in a closed transparent chamber can expand light absorption area and better controlling of growth parameters, thus increasing productivity (Barsanti et al 2006). Flat type photobioreactor use a flat-shaped container culture (Vonshak, 1997). Lee (2001), stated that the development of photobioreactor one of which is expected to lead to the development of flat-type photobioreactor.

Plastic photobioreactor has been known to have the advantage of producing biomass 5. *fusiformis* (Chrismadha, 2009). In addition, plastic is easily to obtain in the market and the price is cheap so it can reduce construction costs in the manufacture of photobioreactor. Plastic photobioreactor is very practical and does not require complicated control system so that the handling easier and simpler.

Light is one of the important parameters in cultures of microalgae and require in adequate intensity. The high exposure of light intensity such as sun light can cause photoinhibition in culture and resulted in a decrease in productivity (Vonshak, 1997). Spirulina requires 10-30 Klux light intensity or about 10-30% of the total intensity of sun light (Vonshak, 1999). Therefore, exposure to direct sun light should be avoided in cultured Spirulina.

METHODS

The study was conducted by using completely randomized design (CRD) consisting of 6 treatments and 4 replications. The treatments were given in the form of sun light filtering of 90% (FI), 80% (F2), 70% (F3), 60% (F4), 50% (F5), and one treatment without sun light filtering (0%) as a control. The percentage indicates the amount of filtering the

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light intensity is reduced. For example 70 % filtering treatment, the sun light that comes will be reduced by 70% so that the sun light received by culture was 30% of the total light coming. This is done by way of shade photobioreactor with paranet 70% (± 5%).

Single culture of *S. fusiform* obtained from Planktonology Laboratory, Limnology Research Center, LIPI-Cibinong. *Spirulina fusiformis* cultured in batch culture in a Zarrouk's medium that has been modified ($FeSO_4.7H_2O$; KH_2PO_4; K_2SO_4; $NaNO_3$; Na_2-EDTA; NaCl; $NaHCO_3$; $MgSO_4$; H_3BO_3; $CuSO_4.5H_2O$; NH_4VO_3 $MnCl_2.4H_2O$; ZnCl; $Te_2(SO_4)_3$;$K_2Ca(SO_4)_4 24H_2O$;$Co(NO_3)2.6H_2O$;$NiSO_4.7H_2O$;$NaMo_3.2HO$

Photobioreactor made by cutting Plastic Roll (width 50 cm) 2.5 meters long and tied at both ends. One side was tied tightly while another side with not too strong to be easily opened when entering a starter or retrieval of data. Preliminary test with that size of plastic can produce flat-shaped photobioreactor container with dimensions of 50 x 100 x 5 cm and a capacity of 40 liters. In this study required 24 pieces flat photobioreactor.

The study begins by entering the 36 liters of medium Zarrouk and 4 liters single culture of *S. fu.siform* into the photobioreactor. Final cell density at the culture was 0,07g/L. Furthermore photobioreactor placed in the open and shaded with paranet appropriate research design. The gases inside the photobioreactor remove every morning, afternoon and evening. In addition it also stirred culture every morning and evening by pulling one end of the photobioreactor (Chrismadha, 2009). The process was done in the Arboretum Padjadjaran culture for 14 days.

During the culture process, measurements of parameters performed such as growth, light intensity, temperature and pH. Growth was measured every second day using Gravimetric methods. A total of 20-50 ml culture of *S. fusiform* filtered with a filter paper and then roasted at a temperature of 60 degrees during the night. The dry weight of the filter paper and *S. fusiform* reduced paper weight is the dry weight of *S. fusiform* (Pandey, 2010; Gao, 2008; Chrismadha 2009 & Vonshak, 1997). Temperature and light intensity measurements performed every day at 09.00; 12:00 and 15:00. pH was measured every two days.

Data growth has been obtained and analyzed to determine the rate of growth, productivity and the effect of sun light filtering treatment against *S. fusiform* productivity levels. Growth rate is calculated with the following formula above (Andersen, 2005; Chrismadha, 2009; Barsanti et a1, 2006 & Vonshak, 1997):

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1} \quad (1)$$

μ : The growth rate (μ/day)
x_1 : Biomass concentration at t_1
x_2 : Biomass concentration at t_2
t_2-t_1 : Range of time (days)

Productivity *S. fusiform* calculated using sun light the following formula (Chrismadha et al, 2006):

$$P = \frac{m_t - m_0}{t} \quad (2)$$

Q : Productivity biomass (g/l/day)
m_t : Biomass concentration on day t (g)
m_0 : Biomass concentration on day 0 (g)
t : Duration of culture (days)

Effect of difference in the percentage of sun light filtering treatment against *S.fusiform* biomass productivity levels were analyzed by Analysis of Variance (ANOVA). If there is a real effect then test followed by Duncan's Multiple Test with a level of 5%.

RESULTS

Research result showed that the *Spirulina fusiformis* only can grow up in photobioreactor which treated by sun light filtering (F1, F2, F3, F4, and F5), whereas in untreated photobioreactor, *S. fusiformis* did not grow. It was signed by the density of *S. fusiformis* cell which only present at sun light filtering treatments. It means that the sun light filtering influential real against to *S. fusiformis* growth (Figure 2). Besides that, sun light filtering is very important to prevent negative impact of direct sun radiation.

Figure 1. Research Condition

The highest grow rate is obtained at 50 % filtering sun light treatment (F5), and the lowest grow rate is obtained at 90% sun light filtering treatment (F1). At the control (0 % sun light filtering), *S. fusiformis gro*wt rate was 0 % (Figure 3).

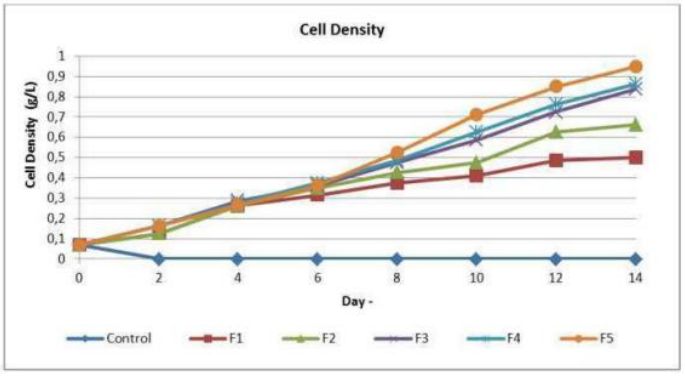

Figure 2. Cell density of *Spirulina fusiformis.*

Table 1. Cell density during research

Treatments	Day - (g/L)							
	0	2	4	6	8	10	12	14
Control (0 %)	0,07	0	0	0	0	0	0	0
F1 (90 %)	0,07	0,125	0,262	0,315	0,375	0,412	0,485	0,5
F2 (80 %)	0,07	0,125	0,262	0,350	0,425	0,475	0,625	0,662
F3 (70 %)	0,07	**0,162**	**0,287**	0,362	0,475	0,587	0,725	0,825
F4 (60 %)	0,07	**0,162**	0,275	**0,375**	0,487	0,625	0,762	0,862
F5 (50 %)	0,07	**0,162**	0,262	0,362	**0,525**	**0,712**	**0,85**	**0,95**

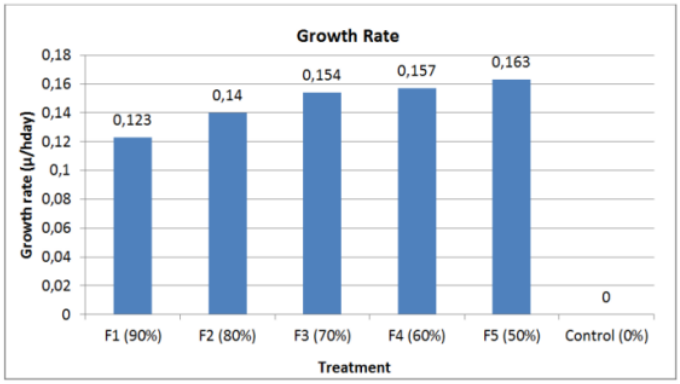

Figure 3. Growth rate of *Spirulina fusiformis*

As a photosynthetic organism, productivity of *S. fusiformis* was extremely influenced by light factor. The result showed that sun light filtering treatment significantly influence productivity of *S. fusiformis.* It is based on the result of ANOVA statistical test which showed the value of $A_{arithmetic}$ (414) was bigger than F_{table} (2,77). It means, there was a

significant dissimilarity of productivity in each treatment. The highest productivity was obtained at F5 treatment (0.057 g/l/day) and the lowest was obtained at control treatment (0 g/l/day). By the productivity of F5 treatment, the photobioreactor in this research can produce up to 18.35 ton/ha/year biomass of *S. fusiformis*.

Generally, commercial productivity of spirulina production in the world is 20-50 ton/ha/year (Carlson et al. 2007). Based on it, although the photobioreactor in this research was made from cheap material and simply contruction but the productivity almost reach that productivity range. It means that the photobioreactor developed in this research effective enough to be use, especially for poor people in devious regions. So it can be used to strengthen food security, especially to against hunger and malnutrition.

Table 2. Productivity of *Spirulina fusiformis*.

Treatment	Productivity	
	(g/L/day)	(ton/ha/year)
Control	0 [a]	0
F1 (10%)	0,031 [b]	8,97
F2 (20%)	0,042 [c]	12,30
F3 (30%)	0,054 [d]	15,64
F4 (40%)	0,057 [d]	16,47
F5 (50%)	0,063 [e]	18,35

It causes the F5 treatment has more energy to run more intensively photosynthetic activity. Light is needed by photosynthetic organism as energy resources to run their metabolism, especially in photosynthetic activity (Campbell et al. 1999). Intensity of light influence to photosynthetic rate (Vonshak 1997 & Nontji 2006). Mean of the highest to lowest intensity of light in a row are 40.88 Klux (F5), 31.64 Klux (F4),13.33 Klux (F3), 4.72 Klux (F2), and 3.21 Klux (F1). Treatment of F5 has highest productivity because this treatment has highest supply of light compared to other treatments.

Table 3. Productivity of *Spirulina fusiformis*.

Time	Light intensity (Klux)					
	Control	Fl	F2	F3	F4	F5
09.00	78,16	2,89	4,54	11,74	27,20	33,26
12.00	98,84	3,37	5,16	15,79	41,29	46,91
15.00	83,57	3,21	4,46	12,47	26,42	42,47
∑	86,86	3,16	4,72	13,33	31,64	40,88

Besides supported by light intensity, high growth of F5 also supported by better pH and temperature condition compared to other treatments. The treatment F5 has the most rapid increase in pH, from 8.7 to 9.42. This condition is good for the growth of spirulina because spirulina is alcalophylic algae, grow well in a pH range of 8-11 and growth optimal at pH 9 (Ciferri, 1983, Sigee, 2005; Pandey et al, 2010 & Vonshak, 1999).

Temperature conditions at F5 was treatment more optimum than other treatments. Spirulina like high temperatures and grows optimally at temperatures range 35-38 °C (Vonshak, 1997; Ciferri, 1988 & Van Eykelenburg, 1980). That optimum temperature was optimally achieved by F5, 29-38 °C. It causes the F5 treatment had a higher rate of photosynthesis than others. An increase in temperature at a certain limit can increase the maximum rate of photosynthesis. It means that within certain limits, the higher temperature conditions photosynthetic rate of microalgae is faster (Masojidek et al, 2004 & Vhonsak 1997).

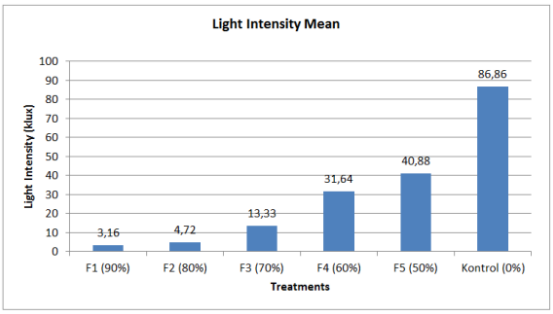

Figure 4. Mean of light intensity.

Despite having the highest productivity, F5 treatment did not continue to show the highest growth during research (Table 1). In the early days of culture, cell density of F5 tended to be lower (Figure I). This is signed by the delay in treatment F5 to achieve the highest cell density. Highest cell density is achieved by F5 on day 8. Delay was caused by photoinhibition that occurred in the early days of culture. Photoinhibition is reduction of the rate of photosynthesis because of light intensity is too high (Barsanti et al, 2006 & Nontji, 2006). Photoinhibition in the F5 treatment occurs because the density of cells in the early days of culture is still low. Low cell density causes the penetration of light into the culture is relatively high (Richmond, 1999; Torzillo, 2003 and Masojidek et al, 2003).

Photoinhibition in the F5 treatment does not occur continuously, but decreased. The decrease occurred due to increase of cell density that decreases light penetration (Richmond, 1999; Torzillo, 2003; & Masojidek et al, 2003). On day 8, the high density of cells in the treatment of F5 was able to reduce light penetration to a level that can be tolerated. This can be seen from cell density of F5 shown the highest cell density from day 8 until the end of the culture period. According to Vonshak (1988), photoinhibition is not permanent because the repair (recovery) will take place when the light intensity decreases.

Control treatment did not show the presence of productivity (Figure 3). This was because *S. fusiform* on the control treatment died. Death occurs due to lack of sun light filtering in the control treatment. It creates extreme light intensity and temperature conditions inside the photobioreactor. Light conditions and extreme temperatures were not able to be tolerated by *S. fusiform*. The absence of sun light filtering can be caused the light intensity in the control treatment was very high, can reach 103 Klux at midday. Based on several studies, it is known that high intensity light such as direct sun light radiation can damage cell structure of spirulina, such as filaments, tricom, as well as DNA and chloroplast thylakoid membrane (Li et al, 2008; Wu et al, 2005; Gao 2008; Gupta 2008 ; Leegood et al, 2000; Hans et al, 2007; & Sechbach 2007).

The absence of sun light filtering also causes the temperature in the control treatment is very high, reaching 46°C. At temperatures above 45°C, the protein in most living organims will be denatured and can lead to damage and lysis tricom on Spirulina (Champbell, 1999 & Ciferri, 1983). This is evidenced by the observation of culture under the microscope showed *S. fusiformis* filament structures that appeared to be empty and most have undergone lysis (Figure 5).

Figure 5. Microscope observation on control treatment show empty filament structure (a) and lysis cell (b).

In this research conducted the protein test content of the biomass produced. The data obtained were compared to the standard quality protein in Spirulina according Vonshak (1997). This test was conducted to determine whether the biomass produced has good quality or not. The test results showed the protein content in each treatment was different (Table 4).

Table 4. Productivity of *Spirulina fusiformis*

Protein content (%)						**Standard** (%) **(Vonshak,1997)**
F1	F2	F3	F4	F5	**Kontrol**	
43,44	48,86	**49,83**	48,53	46,97	0	50-60

Protein content of the highest to the lowest in a row are F3 (49.83%), F2 (48.86%), F4 (48.56%), F5 (46%), F1 (43.44%), and controls (0%) (Table 4.3). The high protein content in F3 treatment (sun light filtering 70%) occurred due to the light conditions at that treatment was in the optimum range. Light intensity on treatment ranged from 11.74 to 15.79 Klux F3 (Figure). Spirulina grow up optimally at the light intensity range of 10-30 Klux (Vonshak, 1997). This conditions make the synthesis of proteins at F5 was better than other treatments.

Treatment of F1 has lowest protein content caused by the availability of light is too low, ranging from 2.89 to 3.37 Klux (Figure). It makes the metabolism is not running optimally at F1. At F5 treatment, low content of protein caused by the supply of light was too high. At the certain level of light intensity the protein content can be decreased due to the high light intensity (Tomaselli et a1 1997). This happens due to the high light intensity conditions form carbohydrates as the first product of photosynthesis is faster than the use of carbohydrates to synthesize the protein (Chrismadha et al, 2009).

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REFERENCES

Ahsan, M.B.H., Hasan, M.R., Hutington, T.C. and Parvin, M. (2008) A Review on Culture, Production and Use of *Spirulina* as a Food for Human and Feeds for Domestic Animal and Fish, *FAO Fisheries and Aquaculture Circular* No. 1034. Rome.

Andersen, Robert. A (2005) Algal Culturing Techniques. Phycological Society Of America-Elsevier Academic Press. USA.

Barsanti, Laura & Gualtieri, Paolo (2006) Algae Anatomy, Biochemistry, and Biotechnology. CRC Press. New York.

Belay, A (2005) Spirulina *(Arthrospira):* Production and Quality Assurance. In: Gershwin, A & Belay, A (ed). Spirulina in Human Nutrition and Health. CRC Press, Taylor & Francis Publishers, London, pp 1-25.

Champbell, N.A., Reece, J.B., & Mitchell, L.G. (1999) Biologi-Jilid Satu. Diterjemahkan oleh Wasmen Manalu. Penerbit Erlangga. Jakarta.

Chrismadha, T., Mardiati, Y., Widoretno, M.R. (2009) Pengaruh Diameter Kolom Fotoreaktor terhadap Pertumbuhan *Spirulina jusijormis* di Tempat Terbuka. Pusat Penelitian Limnologi, LIPI- Cibinong. Bogor.

Chrismadha, T., Mardiati, Y., Widoretno, M.R. (2009). Uji Coba Kultur *Spirulina fusiformis* Dalam Fotoreaktor Kolom Tersirkulasi di Tempat Terbuka. Pusat Penelitian Limnologi, LIPI-Cibinong. Bogor.

Chrismadha, T., Pangabean, T.M., Mardiati, Y. (2006) Pengaruh Konsentrasi Nitrogen dan Fosfor terhadap Pertuinbuhan, Komposisi Biokimia serta Produksi Fikosianin pada *Spirulina fusi%rn1is.* Pusat Penelitian Limnologi, LIPI-Cibinong. Bogor.

Ciferri, Orio. (1983) Spirulina, the Edible Microorganism.*Microbiological Reviews.* Vol. 47. No. 4. p551-578.

Gao, K., Li, P., Watanabe, T & Helbling, E.W. (2008) Combined Effects of Ultraviolet Radiation and Temperature on Morphology, Photosynthesis, and DNA of *Arthrospira (Spirulina) platensis* (Cyanophyta). *J. Phycol.* Vol: 44, pp777-786.

Gupta, Radha., Bhadauriya, Pratiksha., Singh, V.C. & Singh, P.B. (2008) Impact of UV-B Radiation on Thylakoid Membrane and Fatty Acid Profile of *Spirulina platensis. Curr Mikrobiol.* 56:151-161.

Hans, W & Held, W. (2005) Plant Biochemistry. 3th-Edition. Elsevier Academic Press. USA.

Lee, Y.K. (2001) Microalgal Mass Culture System and Methods: Their Limitation and Potential. *Journal of Apllied Phycology.* 13: 307-315.

Leegood, R.C., Sharkey, T.D & Van Caemmerer, Caemmerer. (2000) Photosynthesis: Physiology and Metabolism (Advance in Photosynthesis: Vol 9). Kluwer Academic Publisher. Netherland.

Li, Ping & Gao, Kunshan. (2008) Effects of Solar UV and Visible Radiations on the Spiral Structure and Orientation of *Arthrospira (Spirulina) platensis* (Cyanophyta). *Phycologia.* Vol 47 (6). pp:573- 579.

Masojidek, J., Koblizek, M & Torzillo, G. (2004) Photosinthesis in Microalgae, In: Richmond, Amos (ed) Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Science Publisher. USA.

Masojidek, J., Papdcek, S., Sergejevova, M., Jirka, V., Cerveny, J., Kuncl, J., Korecko, J., Verbovikoval, 0., Kopecky, J., Stys, D & Torzillo, G. (2003) A Closed Solar Photobioreactor for Cultivation of Microalgae Under Supra-High Irradiance: Basic Design and Performance. *Journal oj Applied Phycology.* Vol 15: 239-248.

Nontji, Anugerah, (2006) Plankton Laut. LIPI Press, Jakarta.

Pandey, J. Prakash dan Tiwari, Amit. (2010) Optimization of Biomass Production of *Spirulina maxima. Journal Of Algal Biomass Utilization.* 1(2): 20-32.

Richmond, A & Zou, Ning (1999) Efficient Utilisation of High Photon Irradiance for Mass Production of Photoautotrophic Micro-organisms. *Journal oj'Applied Phycology.* Vol 11: 123-127.

Seckbach, Joseph. (2007) Algae and Cyanobacteria in Extreme Environment. Springer. Netherland.

Setiawan, A., Kardono., Darmawan, R.A., Santoso, A.D., Stani, A.H. & Prasetyadi. (2008). Teknologi Penyerapan Karbondioksida dengan Kultur Fitoplankton pada Fotobioreaktor. Pertemuan Ilmiah Tahunan V Ikatan Sarjana Oseanologi Indonesia. ITB. Bandung.

Sigee, David, C. f2005) Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms In The Freshwater. John Wiley & Sons. England.

Torzillo, G., Pushparaj, B., Masojidek, I & Vonshak, A. (2003) Biological Constrainst in Algal Biotechnology. Biotechnology and Bioprocess Enginerring. Vol. 8: 338-348.

Van Eykelenburg, C. (1980) Ecophysiological studies on *Spirulina platensis.* Effect of Temperature, Light Intensity and Nitrate Concentration on Growth and Ultrastructure. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 46:113-127.

Vonshak, A., Guy, R., Poplawsky, R & Itzhak, 0. (1988) Photoinhibition and Its Recovery in Two Strains of the Cyanobaeterium *Spirulina platensis. Plant and Cell Physiology. Vol 29 L4). 721-726.*

Vonshak, A. (1997) *Spirulina platensis (Arthrospira):* Physiology, Cell- Biology and Biotechnologi. Taylor & Francis Published. London.

Wu, H., Gao, K., Villafane, V.E., Watanabe, T & Helbling, E.W. (2005) Effects of Solar UV Radiation on Morphology and Photosynthesis of Filamentous Cyanobacterium *Arthro.spira platensi.s. Applied and Environmental Microbiology.* Vo1.7:1. Pp5004-501