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Photosynthetic Behavior of Microalgae in Response to Environmental Factors

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1. Introduction

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in a wide range of ecosystems extending from terrestrial to aquatic environment. Environmental factors such as temperature, UV-light, irradiance, drought and salinity are known to affect their photosynthesis.

Photosynthesis was the most sensitive process in microalgae, leading to numerous changes in structure and function of the photosynthetic apparatus under various conditions. The photosynthetic response may be involved in the modification of energy collector complexes, antennas, or reaction centers and in the distribution of excitation energy between the two photo systems (PSII and PSI).

Photosynthesis of micro algae is often inhibited by salt stress (Kirst, 1990). Such an inhibition may be explained by a decrease in PSII activity. Indeed, in the green microalgae, salt stress inhibits PSII activity in *Dunaliella tertiolecta* and *Chlamydomonas reinhardtii* that is associated with a state-2 transition (Gilmour et al., 1985, Endo et al., 1995).

In the cyanobacteria, it has been suggested that the decreased PSII activity in salt-stressed cells is associated with the state-2 transition (Schubert et al., 1993; Schubert and Hagemann, 1990). It has been reported that salt stress significantly inhibit the maximal efficiency of PSII photochemistry in *Spirulina platensis* cells and this inhibition is increased with increasing light intensity (Lu et al., 1999; Lu & Zhang, 1999, 2000).

The decreased maximum quantum yield of primary photochemistry (Fv/Fm), interpreted as photodamage (Powles, 1984; Torzillo et al., 1998; Maxwell & Johnson 2000), may be due to an inactivation of PSII reaction centers, an inhibition of electron transport at both donor and acceptor sides of PSII, and a distribution of excitation energy transfer in favor of PSI (Lu & Vonshak, 1999; Lu et al., 1999; Lu & Vonshak, 2002), increasing the cyclic electron flow around PSI (Gilmour et al., 1982; Joset et al., 1996). This decrease is often identified as an adaptive acclimation process of down regulation of PSII, a protective mechanism that helps dissipate excess energy from the photosynthetic apparatus (Allen & Ort, 2001; Tsonev et al., 2003; Hill et al., 2004; Kramer et al., 2004; Hill & Ralph, 2005).

In *Synechocystis*, it has been reported that the decreased PSII activity by salt stress can be explained the fact that salt stress inhibits the repair of photo-damaged PSII by by concealing

the synthesis of D1 protein (Allakhverdiev et al., 2002). D1 protein of thylakoid membranes was showed as a sensitive protein to environmental stress conditions: under various unfavorable conditions like drought, nutrition deficiency, heat, chemical stress, ozone fumigation as well as UV-B and visible light stresses can influence the turnover of D1 protein (Giardi et al., 1997). In *Synechococcus* cells, salt stress inactivated both PSII and PSI due to the changes in K/Na ratio (Allakhverdiev et al., 2000).

High temperature stress inhibits the function of the oxidizing side of PSII and oxygen evolution is significantly decreased (Havaux, 1993). A loss of the oxygen evolving complex activity is thought to be due to the release of manganese atoms and the dissociation of the manganese stabilizing 33kDa protein from the PSII reaction center complex (Nash et al., 1985; Yamane et al., 1998).

High temperature stress also results in an inactivation of PSII reaction centers (Bukhov & Carpentier, 1990; Toth et al., 2005). In addition, high temperature results in a shift of the redox equilibrium between the primary acceptor plastoquinone (Q_A) and the secondary acceptor plastoquinone (Q_B) (Pospisil & Tyystjarvi, 1999; Toth et al., 2007). Furthermore, high temperature stress induces a dissociation of the peripheral antenna complex of PSII from its core complex (Armond et al., 1980; Wen et al., 2005).

As response to low temperature, Kenya strain of *Arthrospira platensis* was better acclimated than M2 strain by down-regulating its photosynthetic activity through decreasing antenna size and thus reducing energy flux into the photo-systems, decreasing reaction center density and the performance index, thus decreasing the trapping probability and electron transport beyond Q_A^- unchanged and increasing the energy dissipation flux.

Hence, the Kenya strain minimized potential damage on the acceptor side of PSII as compared to the M2 cells. Acclimation to low temperature was accompanied by an improved mechanism for handling excess energy resulting in an enhanced ability to rapidly repair damaged PSII reaction centers and withstand a high photon flux density stress; which was defined as a cross adaptation phenomenon (Vonshak & Novoplansky, 2008).

Increase in pigment in response to decrease in light intensity has long been considered an important and adaptive response because it increases the cellular efficiency of light-harvesting under light -limiting conditions (Richardson et al., 1983; Falkowski & LaRoche, 1991). This so-called photo-adaptive response can be compared to responses to other environmental factors, including temperature, nutrient availability and chemicals that affect cell metabolism and growth (Laws & Bannister, 1980; Rhee & Cotham, 1981; Verity, 1982; Fabregas et al., 1986; Osborne & Geider, 1986; Geider, 1987; Kana & Gilbert, 1987; Kana et al., 1992). Pigmentation such us Chlorophyll-a, is generally decreased under steady state nutrient limitation or transient starvation, or under lowered temperature, however carotenoids often remain high (Young, 1993) under photo-inhibition. Indeed, carotenoids may act as a screening pigment that blocks excess light, as well as scavenging reactive oxygen species, thereby decreasing the damaging effects of intense illumination at low temperatures (Krause, 1993). Furthermore, at low temperature, photosynthetic capacity decreased due to depress activity of ribulose-1,5-bisphosphate carboxylase (Rubisco) (Li et al., 1984; Raven & Geider, 1988).

Then, results of our works dealing with photosynthetic behaviors of the cyanobacteria *Arthrospira platensis* under various conditions, such as salinity and mixotrophic nutrition mode and those of combined effect of temperature, light intensity and C/N ratio on photo-system II photochemistry in *Cosmarium. sp* isolated from Tunisian geothermal source.

2. Effect of salt concentration on growth, fluorescence, photosynthetic activities and pigment content of the cyanobacteria *Arthrospira platensis*

Arthrospira platensis is commercially produced as a nutrient source for health food, feed and pharmaceutical industries, especially in developing countries. These species are representative of a relatively wide group of filamentous cyanobacteria and have been isolated from various habitats, with low to high ionic strength, in salty and alkaline waters. These cyanobacteria have developed different strategies for their adaptation to extreme environmental conditions, such as salinity, which are commonly encountered during biomass production of outdoor cultures.

Under several conditions, the helical filaments of *Arthrospira platensis* became straight after successive generations. These changes occur after pronounced evaporation, therefore salt stress could be considered as the main cause of this transformation.

In this section we investigated the effect of salt concentration on adapted cells of *Arthrospira platensis*, straight morphone in order to explore salty medium. The physiological behavior was evaluated by studying growth, pigment content, photosynthetic activity, change in the distribution of excitation energy between the two photo-systems (PSI and PSII) and the PSII photochemistry (Ben Dhiab et al., 2007).

The increase in salt concentration from 17mM to 500mM enhanced the growth as expressed by the increase of chlorophyll-a content from 52µg mL^{-1} to 70µg mL^{-1} (Fig.1) and a significant increase in phycobilin and carotenoid per Chlorophyll-a contents.
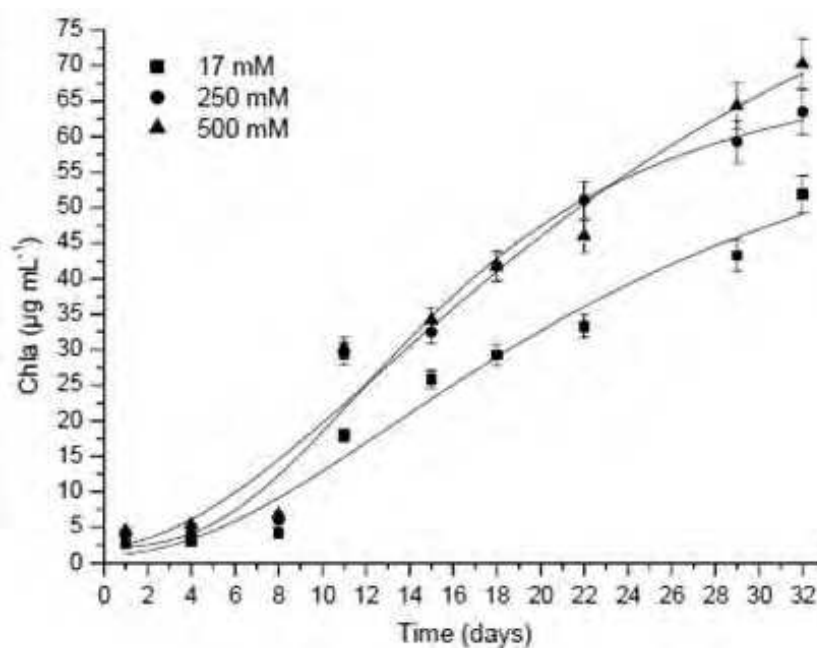

Fig. 1. Chlorophyll-a concentration of *Arthrospira platensis* exposed to different NaCl concentrations. Data are mean ± SEM (n = 3).

The photosynthetic efficiency and the light saturated maximal photosynthetic activity were also increased with the increase of salt concentration; however, dark respiration and compensation points were decreased as shown in (Table 1).

The light state transition regulates the distribution of absorbed excitation energy between the two photo-systems of photosynthesis under varying environmental conditions. In

cyanobacteria, there is evidence of the redistribution of energy absorbed by both chlorophyll and phycobilin pigments. Proposed mechanisms differ in the relative involvement of the two pigment types. Changes in the distribution of excitation energy were assessed using 77K fluorescence emission spectroscopy under excitation of both phycobilin at 570 nm and chlorophyll at 440 nm.

Medium NaCl concentration	α	P_c	R_d	P_m
17 mM	5.79 ± 0.97	11.61 ± 4.47	72 ± 4.07	526.32 ± 2.95
250 mM	7.55 ± 0.60	7.62 ± 1.49	43.2 ± 0.00	864.00 ± 6.11
500 mM	6.46 ± 0.72	7.60 ± 3.15	50.4 ± 3.05	623.52 ± 6.11

Table 1. Photosynthetic parameters measured after 15 days of growth of the various cultures (P_m: light saturated maximal photosynthetic activity (µmol O_2 h^{-1} mg^{-1}chl), α: initial slope at the P-I curve (µmol O_2 h^{-1} mg^{-1}chl/µmol photons m^{-2} s^{-1}); Rd: dark respiration (µmol O_2 uptake h^{-1} mg^{-1} chl); Pc: compensation point (light intensity in µmol photons m^{-2} s^{-1} where no net oxygen uptake or evolution was observed)
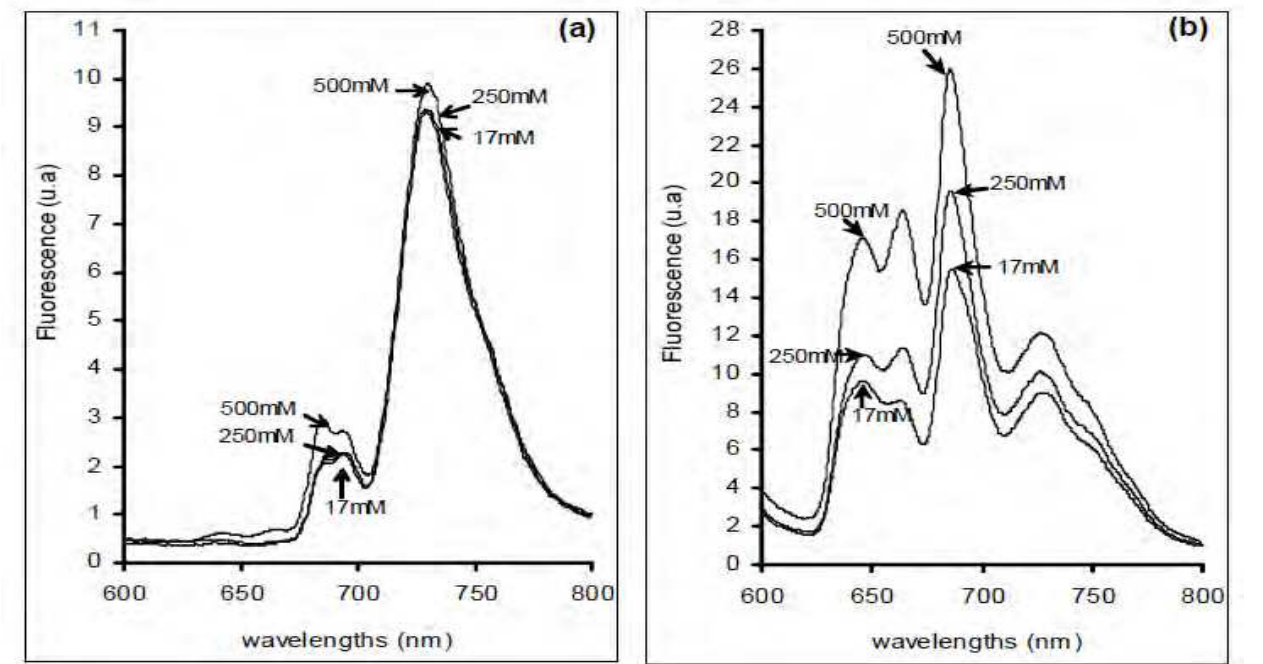

Fig. 2. Fluorescence emission spectra of excitation at 440 nm (a) and of excitation at 570 nm (b) on *Arthrospira platensis* exposed to different NaCl concentration. Spectra are normalized at 800 nm

The ratios between the peak heights at 685 or 695 and 730nm assigned respectively to Photo-system II (PS II) and photo-system I (PS I) (F685/F730) or (F695/F730) were used to obtain qualitative characteristics. These ratios are considered as a relative indicator of the distribution of excitation energy between PSII and PSI and therefore as an indicator of the state of the cells. The increase of NaCl concentration to 500 mM was accompanied with an enhancement of PSII activity per report to PSI under excitation of both chlorophyll and phycobilin (Fig 2).

The ratio between the heights of the peaks at 600 nm and 440 nm, calculated from the emission spectra of PSII at 695 nm (F695,600/F695,440), increased simultaneously with salt concentration of the growth media (Fig 3-a). This effect can be attributed to an increase of

the energy transfer between phycobilisomes and PSII, which therefore enhanced the PSII fluorescence emission peak at 695 nm. This may also explain the elevated F695/F730 ratio in salt adapted cells of *Arthrospira platensis*. Additionally, the highest phycobilin/Chlorophyll-a ratio in salt adapted cultures allows the increase of light absorption by phycobilisomes which therefore increases PSII/PSI ratios.
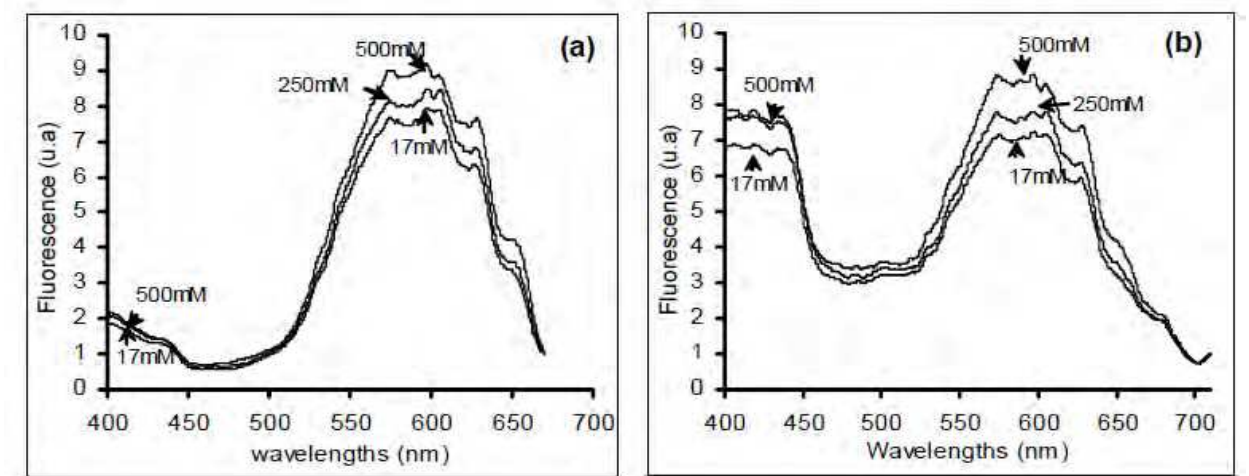

Fig. 3. Excitation spectra of PSII emission at 695 nm normalized at 670 nm (a) and PSI emission at 730 nm normalized at 710 nm (b) on *Arthrospira platensis* exposed to different NaCl concentrations

A direct energy transfer from phycobilisomes to PSI was also observed as shown by the ratio between the heights of the peaks at 600 nm and 440 nm calculated from emission spectra of PSI at 730 nm (F730,600/F730,440) (Fig 3-b). These ratios were close to unity, indicating that the cross section of energy transfer from phycobilisomes and chlorophyll-a to PSI was probably equivalent. These ratios remained relatively unchanged with the increase of NaCl concentration in growth media, resulting in a constant fluorescence emission of PSI. Data of maximal efficiency of PSII photochemistry were not in agreement with the above mentioned results. Indeed, Fv/Fm ratio decreased in salt adapted cultures. However, the trapping flux per PSII reaction center (TR_0/RC) and the probability of electron transport beyond Q_A showed change neither at the donor nor at the acceptor sides of PSII. In plants, Fv/Fm was well defined as an index of the maximal photochemical efficiency of PSII (Bjorkman & Demming, 1987). But this interpretation depended on both F_0 and F_v originating predominantly from sides of PSII (Table 2)

Medium NaCl concentration	Fv/Fm	Ψ_0	TR_0/RC
17 mM	0.46 ± 0.03	0.43 ± 0.08	2.96 ± 0.13
250 mM	0.44 ± 0.03	0.43 ± 0.07	2.83 ± 0.25
500 mM	0.40 ± 0.04	0.41 ± 0.08	2.87 ± 0.18

Table 2. Variable fluorescence parameters measured after 15 days of growth in media with different NaCl concentration; Fv/Fm: maximal efficiency of PSII photochemistry; Ψ_0: probability of electron transport beyond Q_A; TR_0/RC: trapping flux per PSII reaction centre

This assumption was not valid for cyanobacteria (Buchel & Wilhem, 1993; Papageorgiou & Govindjee, 1968; Papageorgiou, 1996; Schreiber et al., 1986), since phycobilin fluorescence interfered with Chlorophyll fluorescence leading to an increase of F_0, and therefore to a

decrease of Fv/Fm values. Consequently, PSII contributed only a small proportion of total chlorophyll (Campbell et al., 1998). These data showed that phycobilin content, compared to the chlorophyll content as assessed by (phycobilin/Chlorophyll-a) ratio, increased with salt concentration and therefore affected the Fv/Fm measured values.

Several works studying the effect of salt concentration on physiological behavior of *Arthrospira platensis* showed different results. Vonshak et al., (1988, 1995, 1996), Zeng & Vonshak (1998), Lu et al. (1998, 1999) and Lu & Vonshak (1999, 2002) have shown that an increase in salt concentration led to the decrease of the specific growth rate, photosynthetic efficiency, maximum rate of photosynthesis, phycobilin/Chlorophyll-a ratio and PSII activity. Conversely, dark respiration activity, compensation points and PSI activity were increased. These contradictory results might be attributed to different genetic and environmental factors. Indeed, according to Berry et al. (2003), bioenergetic processes in the cytoplasmic membrane, the thylakoid membrane and the cytoplasm exhibited special adaptation strategies of strains like *Arthrospira platensis* which were mainly realized by using available components from the "tool-box" with different expression levels. In this study, the Compere strain of *Arthrospira platensis* was used, whereas Lu & Vonshak (1999, 2002) and Pogoryelov et al. (2003) have used, respectively, the M2 and Mayse strains.

Changes in the morphology of the trichome (from the helicoidal to the straight form) seem to be accompanied with modifications of physiological behavior of *Arthrospira* in response to the increase of NaCl concentration in growth media. Indeed, according to Jeeji Bai (1985) and Lewin (1980), the comparative behavior of the two morphones (straight and helicoïdal) in pure cultures showed that NaCl addition over and above the basal level inhibits the growth of the helicoidal morphone, while the straight morphone's growth behavior remained unaffected.

Minor variations in culture conditions might induce differences on physiological responses. Indeed, in our study a light intensity of 20 µmol photon m^{-2} s^{-1} was used, which was lower than the one used by Lu and Vonshak (1999, 2002: 50 µmol photon m^{-2} s^{-1}) and Pogoryelov et al. (2003: 60 µmol photon m^{-2} s^{-1}). These experimental light conditions appeared to be appropriated to maintain growth and full activity of the cells under elevated salinity conditions. Indeed, Zeng & Vonshak (1998) have shown that, at a higher light intensity, growing cells showed lower photosynthetic activity after photo- inhibition under salinity stress, compared with cells growing under lower light intensity conditions. In addition, it has been observed that a 12 h salt stress inhibits electron transport at both the donor and acceptor sides of PSII. However, 2 weeks-old adapted cells showed a down-regulation of PSII reaction centers without any inhibition of electron transfer on the donor and acceptor sides of PSII (Lu & Vonshak, 2002). Moreover, the effect of salt stress on PSII function in *Arthrospira* cells might be attributed to a direct interaction of high salt with PS II or more complex interaction through unknown cell components, which remain to be studied further. Indeed, the time course of adaptation to salinity stress induced different results.

3. Combined effect of light intensity and glucose concentration on *Arthrospira platensis* growth and photosynthetic responses

Arthrospira platensis is a photosynthetic cyanobacterium which is able to convert the energy of sunlight into chemical compounds usable by the cell to fix carbon dioxide and release oxygen. This cyanobacterium was also shown to be able of using organic carbon sources in heterotrophic and mixotrophic culture conditions. (Marquez et al., 1993; Chen et al., 1996;

Zhang et al., 1999; Vonshak et al., 2000; Chojnacka & Noworyta, 2004; Lodi et al., 2005; Andrade & Costa 2007). Mixotrophic growth offers a possibility of greatly increasing microalgal cell concentration in batch culture (Richmond, 1988; Marquez et al., 1993, 1995; Zhang et al., 1999). In mixotrophic growth, there are two distinctive processes within the cell, photosynthesis and aerobic respiration. The former is influenced by light intensity and the latter is related to the organic substrate concentration.

The interaction of light and glucose on specific growth rate was found to follow multiplicative growth kinetics (Chojnacka & Noworyta, 2004). The level of light intensity and glucose concentration and their interaction may influence both autotrophic (photosynthesis) and heterotrophic (oxidative metabolism of glucose) processes and therefore influence cell growth.

In this section, we expose primarily the combined effects of light intensity and glucose concentration on maximal biomass concentration, maximum specific growth rate, maximum net photosynthetic rate, and dark respiration rate and secondly comparative analysis on growth and photosynthetic responses between photoautotrophic and mixotrophic cultures (Ben Dhiab et al., 2010).

The effect of light intensity and glucose concentration on growth and photosynthesis was investigated using designs of response surface modelling (RSM) as shown in Table 3.

Experiment	Factors		Responses			
	Light (μmol photons m^{-2} s^{-1})	Glucose (g L^{-1})	Maximal Biomass X_{max} (g L^{-1})	Maximal specific growth rate (μ) (day^{-1})	Net Photosynthesis (P_n) (μmol O_2 mg Chl^{-1} h^{-1}	Dark respiration (R_d) (μmol uptake O_2 mg Chl^{-1} h^{-1})
1	50	0.5	0.51	0.28	43.77	45.85
2	100	0.5	0.44	0.24	48.56	41.60
3	150	0.5	0.73	0.43	84.32	46.76
4	50	1.5	0.73	0.33	46.91	45.85
5	100	1.5	0.80	0.19	47.61	50.87
6	150	1.5	0.83	0.49	135.79	46.16
7	50	2.5	0.85	0.35	49.21	50.74
8	100	2.5	0.91	0.37	67.78	63.27
9	150	2.5	1.33	0.49	132.7	65.00
10	100	1.5	0.86	0.23	67.83	52.93
11	100	1.5	0.84	0.23	65.29	39.94

Table 3. Maximal specific growth rate (μ_{max}), maximal biomass concentration (X_{max}), net photosynthetic rate (P_n) and dark respiration rate (R_d) for various culture conditions of light intensity and glucose concentration.

Analysis of the results was performed by MODDE. 7.0. The effect of each factor and their interactions was obtained by ANOVA with confidence interval of 90%.

Growth was characterized by two responses: maximum biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}), whereas photosynthesis was evaluated by maximum net photosynthetic rate (P_n) and dark respiration rate (R_d).

The results showed that *Arthrospira platensis* grew in the presence of organic substrate (glucose) in the light. Independently of light intensity and glucose concentration, rates of the instantaneous relative growth, net photosynthesis, and dark respiration demonstrated two different phases (Fig. 4).
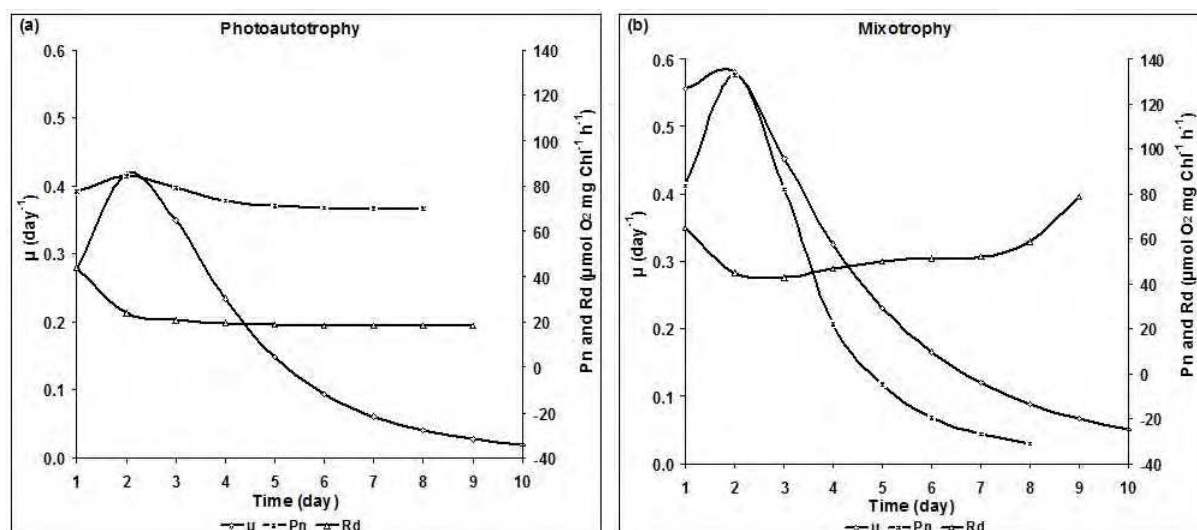

Fig. 4. Rates of the instantaneous relative growth, net photosynthesis, and dark respiration under photoautotrophic (a) and mixotrophic (b) conditions at a light intensity of 150 μmol photons m^{-2}s^{-1}

The first phase occurred during the first 3 to 4 days. It was characterized by the highest rates of instantaneous relative growth and net photosynthesis, as well as the preponderance of photosynthetic activity even in mixotrophic cultures, as seen by the increase in pH until the third day. This result might be supported by the data reported by Yang et al. (2000) who found that light was the major source for ATP production in the early phase of mixotrophic cultivation.

The second phase occurred from the fourth day onwards. It was characterized by the decrease of the instantaneous relative growth rates. In photoautotrophic cultures, rates of maximal net photosynthesis and dark respiration were maintained at constant values which are lower than those observed during the first phase. However, in mixotrophic cultures, net photosynthetic rate was reduced to negative values and dark respiration rate increased. Thus, we suggest that metabolic activity was based essentially on photoheterotrophy. This suggestion is supported by the decrease of pH during this phase, due to the release of carbon dioxide, caused by the heterotrophic component of mixotrophic metabolism as reported by Hase et al. (2000).

These results are in contrast with those observed by Chen & Zhang (1997) who showed that heterotrophic metabolism dominated in the first phase, then decreased subsequently as the glucose was consumed, and later photosynthesis became predominant. The same comment was signaled by Andrade & Costa (2007). Indeed, these suggestions were based only on the glucose consumption and phycocyanin content. Our results showed effectively that the total glucose in the medium was consumed during the first 3 days, considered as the photoautotrophic phase and characterized by the highest net photosynthetic rate. Thus, we hypothesise that glucose was stored as reserve carbohydrate (glycogen) to be metabolized in the second phase considered as heterotrophic. Indeed, Pelroy et al. (1972) showed in

Synechocystis sp. that exogenous glucose is mainly stored as glycogen under illumination before being metabolized for the maintenance of cells (Yang et al., 2000). Our hypothesis is supported by results of Martinez and Orus (1991) who noted that respiratory rate was noticeably enhanced in mixotrophic cultures, reflecting the increasing rate of glucose metabolism after the induction of glucose uptake ability. Moreover, in the second phase and additional to the decrease of the net photosynthetic rate, we noted a reduction of the photochemical efficiency of photosystem II accompanied by lower electron transport rate. Therefore, organic carbon sources reduced the photosynthetic efficiency in this phase, and the enhancement of biomass of *Arthrospira platensis* implied that organic sources had more pronounced effects on respiration than on photosynthesis. These conclusions are in agreement with results obtained with *Phaeodactylum tricornutum* under mixotrophic culture (Liu et al., 2009).

All the above results confirmed that, in the second phase, *Arthrospira platensis* might use and metabolize glucose and then shift to the heterotrophic nutrition mode. Comparison between mixotrophic and autotrophic cultures showed that the former were characterized by the highest values of the instantaneous relative growth rates and maximal biomass concentration. These results are consistent with those obtained by Marquez et al. (1993, 1995), Vonshak et al. (2000), Zhang et al. (1999), and Andrade and Costa (2007). Marquez et al. (1993) and Hata et al. (2000) suggested that in mixotrophic culture, a simultaneous uptake of organic compounds and CO_2 takes place as carbon sources for cell synthesis, and it is then expected that CO_2 will be released via respiration and will be rapidly trapped and reused under sufficient light intensity. Thus, mixotrophic cells acquire the energy by catabolizing organic compounds via respiration and converting light energy into chemical energy via photosynthesis (Hata et al., 2000).

Effectively, both photosynthetic and dark respiration rates were the highest in mixotrophic cultures, as observed by Vonshak et al. (2000) in *Arthrsopira platensis*, Kang et al. (2004) in *Synechococcus sp.*, Yu et al. (2009) in *Nostoc flagelliforme*, and Orus et al. (1991) in *Chlorella vulgaris*. The biomass gain recorded in mixotrophic cultures was achieved in the second phase characterized by the nutritive salt reduction and the decrease of the available photons for cells as a consequence of shading caused by the increase in cell density. Such conditions seem to stimulate heterotrophic growth which gives the possibility to increase biomass concentration (Chen et al., 1996; Chen & Zhang, 1997). Martinez et al. (1997) indicated in *Chlorella pyrenoidosa* that light contributes the energy needed for growth and cell maintenance, while glucose is used for the formation of biomass.

Furthermore, the results of Yu et al. (2009) demonstrated that, during the first 4 days, the cell concentration in mixotrophic culture of *Nostoc flagelliforme* was lower than the sum of those in photoautotrophic and in heterotrophic culture. However, from the fifth day, the cell concentration in mixotrophic culture surpassed the sum of those obtained from the other two trophic modes. The reason for this phenomenon requires further investigation.

Growth and photosynthetic activity of mixotrophic cultures were evaluated with respect to light intensity and glucose concentration using response surface methodology as shown in Fig 5. The experimental design showed that polynomial models dependent on light intensity and glucose concentration could describe relatively accurately the maximal biomass concentration, maximum specific growth rate, and maximum net photosynthetic rate.

The results showed clearly that all these responses were influenced by both factors. Furthermore, the interaction of both factors showed that at low light intensity, glucose had a low effect on maximum biomass concentration and maximum net photosynthetic rate.

However, at the highest light intensity (150 µmol photons m^{-2}s^{-1}), the effect of glucose was positive and the responses were more sensitive to it. The effect of this organic carbon substrate might be attributed to the protective role of glucose or to the shift in light intensity at which photo-inhibition occurs, as explained by Chojnacka & Marquez-Rocha (2004). Thus, cells growing heterotrophically might use part of the O$_2$ produced by cells growing photoautotrophically, decreasing dissolved oxygen concentration; this can help reduce photooxidative damage. As has been observed in the response surface plot, the maximum biomass concentration (1.33 gL^{-1}) was obtained at the highest light intensity (150 µmol photons m^{-2}s^{-1}) and glucose concentration (2.5 gL^{-1}). The same conditions improved maximum specific growth rate (0.49 day^{-1}) and maximum net photosynthetic rate (139.89 µmol µmol O$_2$ mg Chl^{-1} h^{-1}). Conditions favouring high biomass production and maximum net photosynthetic rate were, however, not optimal. Indeed, the optimal conditions of light intensity and glucose concentration are not achieved in the experimental range used in this
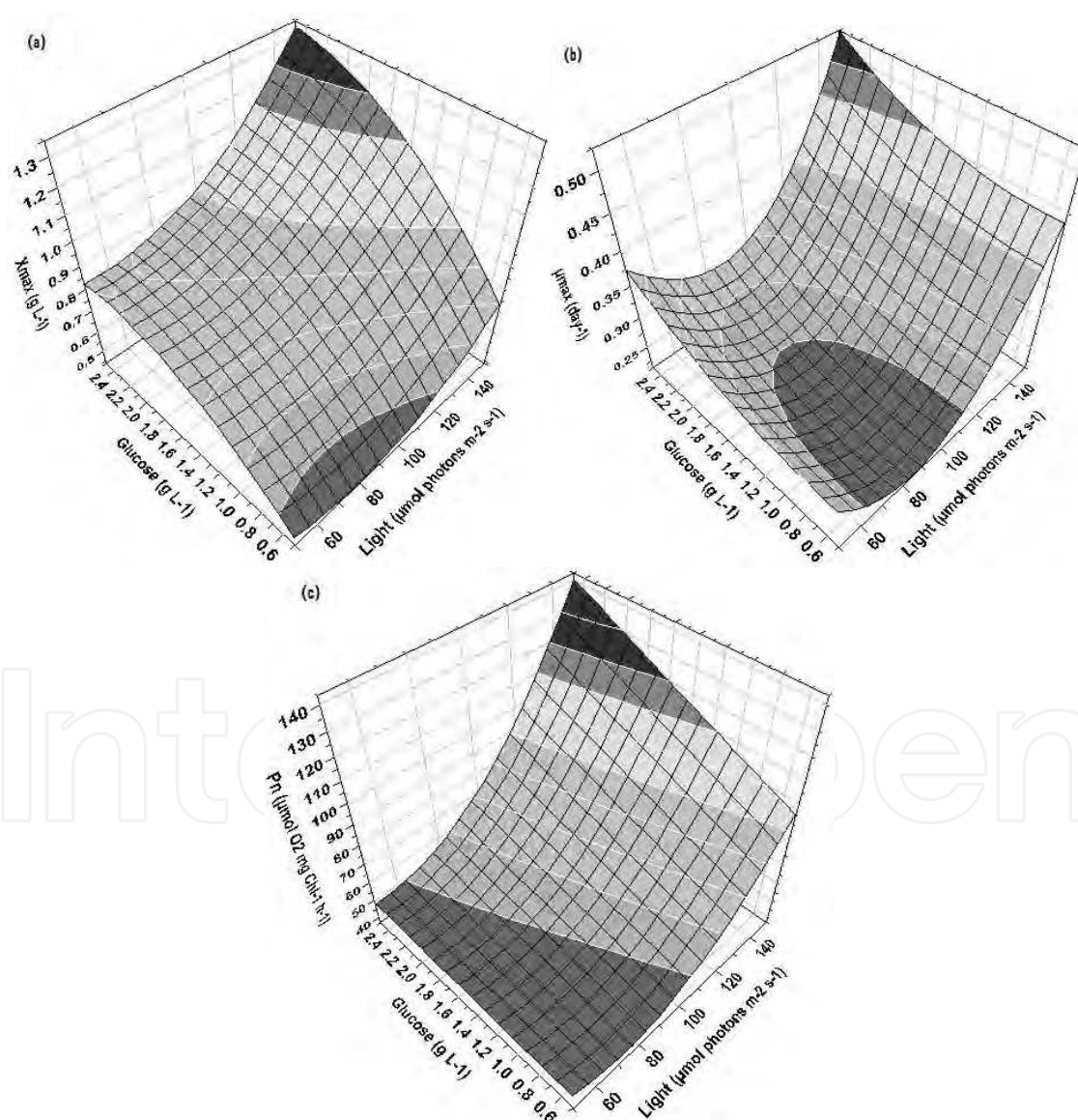

Fig. 5. Response surface plot vs light intensity and glucose concentration for maximal biomass concentration (a), maximal specific growth rate (b) and net photosynthesis (c).

study. Therefore, further studies that extend the experimental range of light intensity and glucose concentration might be required to reveal optimal conditions that maximize growth and photosynthesis of mixotrophic cultures. For the same species, Zhang et al. (1999), using a number of mathematical models, found that the optimal initial glucose concentration and light intensity were 2.5 g L^{-1} and 48 μmol photons m^{-2} s^{-1} (4 klx), respectively. However, Chojnacka (2003) determined optimal growth parameters to be 2.5 gL^{-1} glucose concentration and 126 μmol photons m^{-2}s^{-1} (10.5 klx) light intensity. This difference in the optimal light intensity, as commented by Chojnacka & Marquez-Rocha (2004), could be due to the different methods of light intensity measurement and distribution of cells inside the culture vessels. Considering all the findings drawn from the experimental design, it is also recommended that data from batch cultures should be further examined to develop much more accurate models.

4. Combined effect of temperature, light intensity and C/N on photo system II in *Cosmarium. sp* isolated from Tunisian geothermal using polyphasic rise of fluorescence transients

Hot spring microalgae are exposed to daily and seasonal fluctuations in temperature and light, which are the major factors determining photosynthetic and growth rates. These factors intensities may disturb the balance between the absorption of energy through photosynthesis and the ability to utilize this energy. Such conditions may cause higher excitation pressure on PSII that result in damage to the photosynthetic apparatus (Huner et al., 1998; Yamamoto, 2001; Wilson et al., 2006).

Within the photosynthetic apparatus, photo-system II (PSII) is the most sensitive component of the electron transport chain (Cajanek et al., 1998). Damage to PSII is often the first manifestation of stress. Among partial reactions of PSII, the oxygen evolving complex (OEC) is particularly heat sensitive (Georgieva et al., 2000).

Numerous studies have shown that high temperature stress has various effects on PSII function.

Several major regulatory mechanisms may be involved in the protection of the PSII apparatus from photo-damage under stress conditions: photochemical processes related to electron transport, non photochemical processes by which excess energy is dissipated as heat fluorescence or transferred to other systems, and modification in D1 protein turnover under excess energy stress (Prasil et al., 1992; Campbell et al., 1998; Huner et al., 1998; Melis, 1999; Adams et al., 2001; Yamamoto, 2001; Tsonev & Hikosaka, 2003).

Polyphasic rise in Chlorophyll-a fluorescence (OJIP test) has been used as a tool to evaluate modifications in PSII photochemistry in a wide range of studies, not only in higher plants (Tomek et al., 2001; Ban Dar & Leu, 2003; Force et al., 2003; Zhu et al., 2005; Strauss et al., 2006) but also in algae (Hill et al., 2004; Hill & Ralph, 2005; Kruskopf & Flynn, 2006) and cyanobacteria (Strasser et al., 1995, 2004; Lu & Vonshak, 1999; Lu et al., 1999; Qiu et al., 2004; Lazar, 2006). This tool has been used in a variety of studies, including structure and function of the photosynthetic apparatus, characterization of vitality and physiological condition, and selection of species tolerant to stress conditions (Hermans et al., 2003, Goncalves & Santos, 2005).

Fluorescence induction kinetics (Kautsky curve) of all photosynthetic organisms show a polyphasic rise between initial (F$_0$) and maximum (F$_m$) fluorescence yields (Schreiber & Neubauer, 1987; Strasser et al., 1995; Srivastava et al., 1999). These phases were designed as O, J, I and P, and can be visualized using a logarithmic time scale. By monitoring

fluorescence transients and quenching, it is possible to obtain information on the absorption, transfer and dissipation of energy by PSII.

In this section we study the response of photo-system II under combined environmental factors of temperature, light intensity and Carbon/Nitrogen ratio in *Cosmarium sp* isolated from Tunisian hot spring using Polyphasic rise of fluorescence transients.

Analysis of fluorescence transients in *Cosmarium sp* might be provide information about changes taking place in the structure, conformation and functional of the photosynthetic apparatus, especially in PSII under these environmental factors.

The increase of temperature from 20°C to 60°C causes a significant decrease in the minimal (F_0) and maximal (F_m) fluorescence (Fig 6). A decrease in the fluorescence yield in *Cosmarium* cells can be attributed to an inhibition of electron flow at oxidizing site of PSII (Lu & Vonshak, 2002). The decrease in F_m and fluorescence at J, I, P may be due to two reasons, first by inhibition of electron transport at the donor side of the PSII which results in the accumulation of P680+ (Govindjee, 1995; Neubauer & Schreiber., 1987) and second due to a decrease in the pool size of Q_A^-.

The same results were observed in *Synechocystis* cells exposed to Sb at 10 mg L-1. In fact F_m was decreased and the shape of J–I–P phase became flat with the increase of Sb concentration, indicating that PSII were partially inactivated and could not be closed, and the reduction of PQ (non-photochemical phase) was inhibited (Zhang et al., 2010). In *Spirulina platensis*, Zhao et al. (2008) showed that F_m decreased but F_0 increased significantly with increasing temperature. A loss of the JI-phase at higher temperatures suggests that heat stress resulted in the destruction of the oxygen-evolving complex which may be due to a loss of the manganese cluster activity.

The reduction in the J and P steps observed in the Kenya strain of *Arthrospira platensis* grown at low temperature reflects a bigger decline in the concentration of Q_A, Q_B^{2-}, and plastoquinol (PQH_2) and therefore a decrease in the accumulation of reduced Q_A (Govindjee, 2004; Zhu et al., 2005; Lazar 2006) as well as a decrease in the concentration of active reaction centers (Govindjee, 2004; Zhu et al., 2005; Lazar, 2006).

At the donor side, the quenching effect of the variable fluorescence yield at J, I and P was also ascribed to the deterioration of the water splitting system (Strasser, 1997).

Moreover, the efficiency of the water-splitting complex on the donor side of PSII as expressed from (F_v/F_0) (Schreiber et al., 1994) is the most sensitive component in the photosynthetic electron transport chain. In this study the increase of temperature from 20°C to 60°C decreased this ratio from 2.71 to 0.14. This decrease results from photosynthetic electron transport impairment as indicated by Pereira et al. (2000). The same results were also observed in barely under salt concentration (Kalaji et al., 2011) and in *Synechocystis sp.* exposed to Sb (Zhang et al., 2010).

Area over the fluorescence induction curve between F_0 and F_m is proportional to the pool size of the electron acceptor Q_A on the reducing side of PSII. If the electron transfer from reaction center to quinone pool is blocked, this area will be dramatically reduced (Strasser et al., 2000). As compared to the initial inoculums, the area over the fluorescence curve was decreased with increasing temperature at 60°C to reach ratios of 0.28 and 0.65. However these ratios were augmented to 3.5 and 4.24 times in the lowest temperature at 20°C. This decrease in area over the fluorescence curve suggests that high temperature inhibits the electron transfer rates at the donor side of PSII. This inhibition becomes most considerable under the interaction of the highest temperature and light intensity (60°C/130µmol photons m-2 s-1) which lead to the blockage of the electron transfer from reaction center to quinone
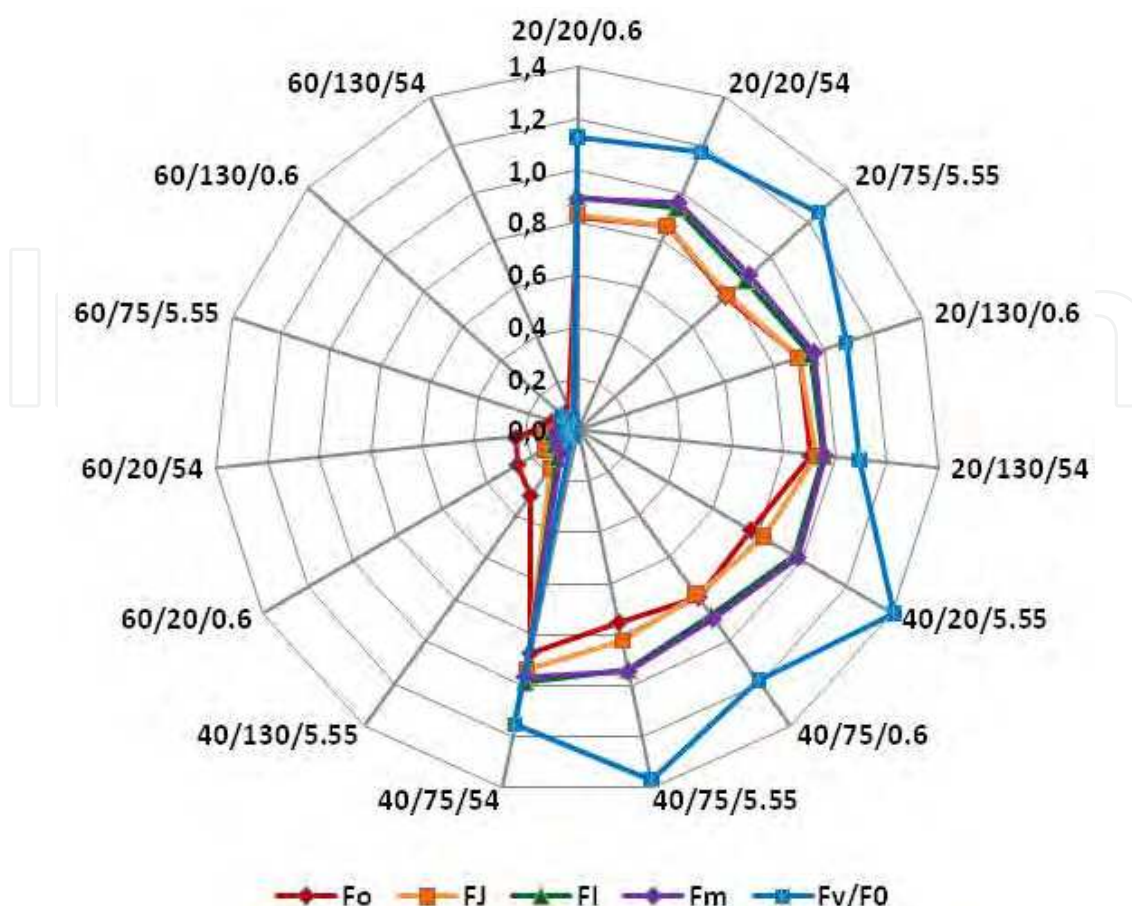
Fig. 6. Spider plot of parameter of fluorescence intensities responses of the OJIP steps and F_v/F_0

pool. These results are in agreement with those of Mehta et al. (2010) which showed an inhibition in the electron transfer rates at the donor side of PS II in *Triticum aestivum* leaves, treated with 0.5 M NaCl.

To localize the action of high temperature in electron transport chain on acceptor side of PSII, the kinetics of relative variable fluorescence (V_j), was calculated. V_j is equivalent to (F_j-F_0/F_m-F_0). F_j is the fluorescence at J step at 2 ms, relative variable fluorescence (V_j) at 2 ms for unconnected PS II units, equals to the fraction of closed RCs at J step expressed as proportion of the total number of the RCs that can be closed (Force et al., 2003). Efficiency with which a trapped exciton can move an electron in to the electron transport chain further than Q_A^- ($\Psi_0 = ET_0/TR_0$) was also measured.

Increasing temperature from 20°C to 60°C provoke an increase of V_j from 85% to 147% and a decrease of Ψ_0 from 112% to 62%. Thus, revealed a loss of Q_A^- reoxidation capacity and an inhibition of electron transport at the acceptor side of PSII and also beyond Q_A^-. The same results were showed in wheat leaves (Mehta et al., 2010), *Spirulina platensis* (Lu & Vonshak, 2002) and *Synechocystis sp.* (Zhang et al., 2010) treated respectively with 0.5 M NaCl, 0.8M NaCl and antimony potassium tartrate (Sb).

Results of specific energy fluxes demonstrated that an increase of temperature from 20°C to 60°C provoked the raise of the effective antenna size per reaction center (ABS/RC) (from 33% to 74.5%), the trapping energy flux per reaction center (from 78% to 493%) and dissipated energy flux per reaction center (from 13% to 936%).
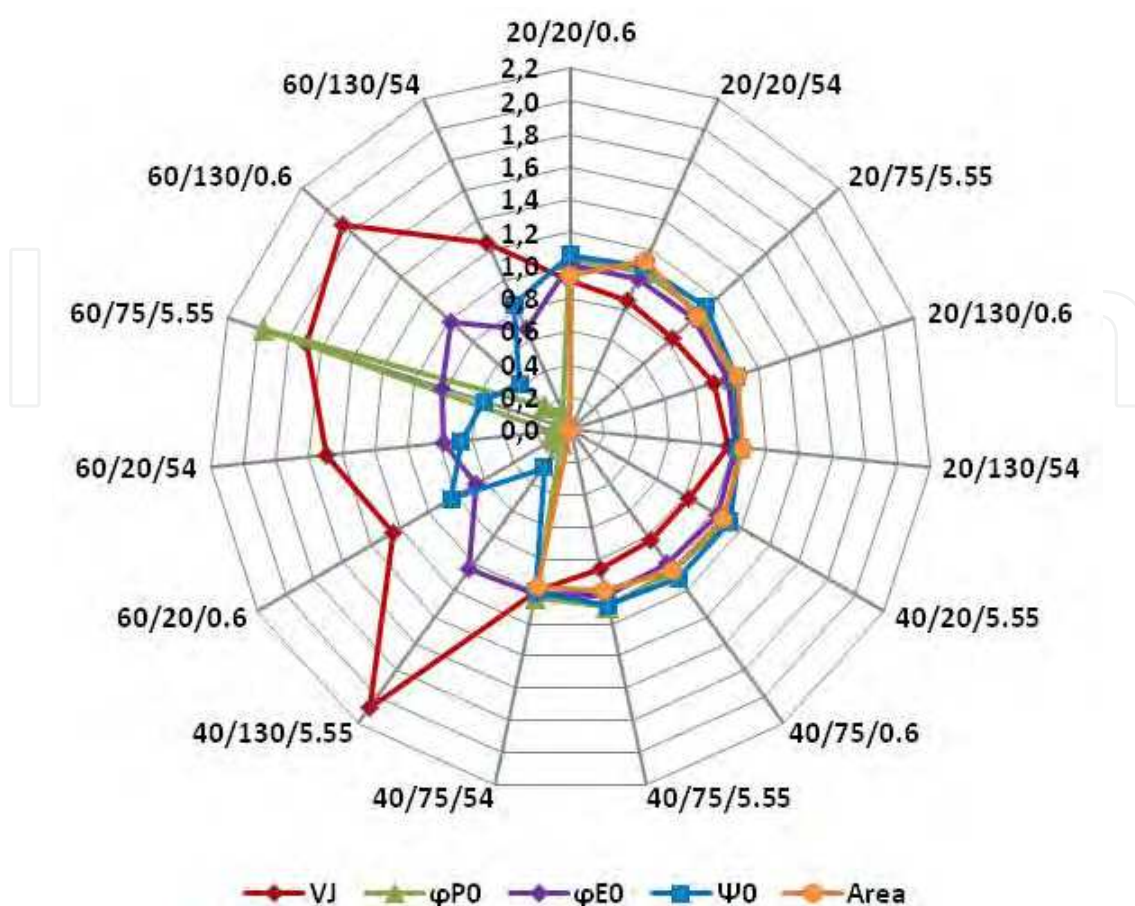
Fig. 7. Spider plot of parameter of quantum efficiencies (φ_{P0}, φ_{E0}, Ψ_0), area, and relative variable fluorescence intensity at the J-step (V_j).

The ratio of ABS/RC demonstrates average antenna size and expresses the total absorption of PS II antenna chlorophylls divided by the number of active (in the sense of Q_A reducing) reaction centers. Therefore, the antenna of inactivated reaction centers are mathematically added to the antenna of the active reaction centers. Consequently, the increase of this ratio was justified by an inactivation of some active reaction centers.

TR_0/RC ratio represents the maximal rate by which an exciton is trapped by the RC resulting in the reduction of Q_A. So it refers only to the active (Q_A to Q_A^-) centers (Force et al. 2003). The increase in this ratio indicates that all the Q_A has been reduced but it is not able to oxidize back due to stress, as a result the reoxidation of Q_A^- is inhibited so that Q_A cannot transfer electrons efficiently to Q_B.

DI_0/RC corresponds to the ratio of the total dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs. Dissipation may occurs as heat, fluorescence and energy transfer to other systems. It is influenced by the ratios of active/inactive RCs. The ratio of total dissipation to the amount of active RCs increased (DI_0/RC) due to the high dissipation of the active RCs.

These ratios conclusively describe that the number of inactive centers have increased due to high temperature in *Cosmarium sp*. These results were confirmed by the decrease in the concentration of active PSII reaction centers (RC/CSm) from 315% to 5% at 60°C, resulted in a similar decrease in maximum quantum yield for primary photochemistry (φ_{P0}) and in the quantum yield of electron transport (φ_{E0}) respectively from 239% to 50% and from

264% to 33%. Furthermore the increase of temperature from 20°C to 60°C provokes the decrease in the performance index (PI$_{ABS}$) from 2361% to 122% which indicates the sample vitality.
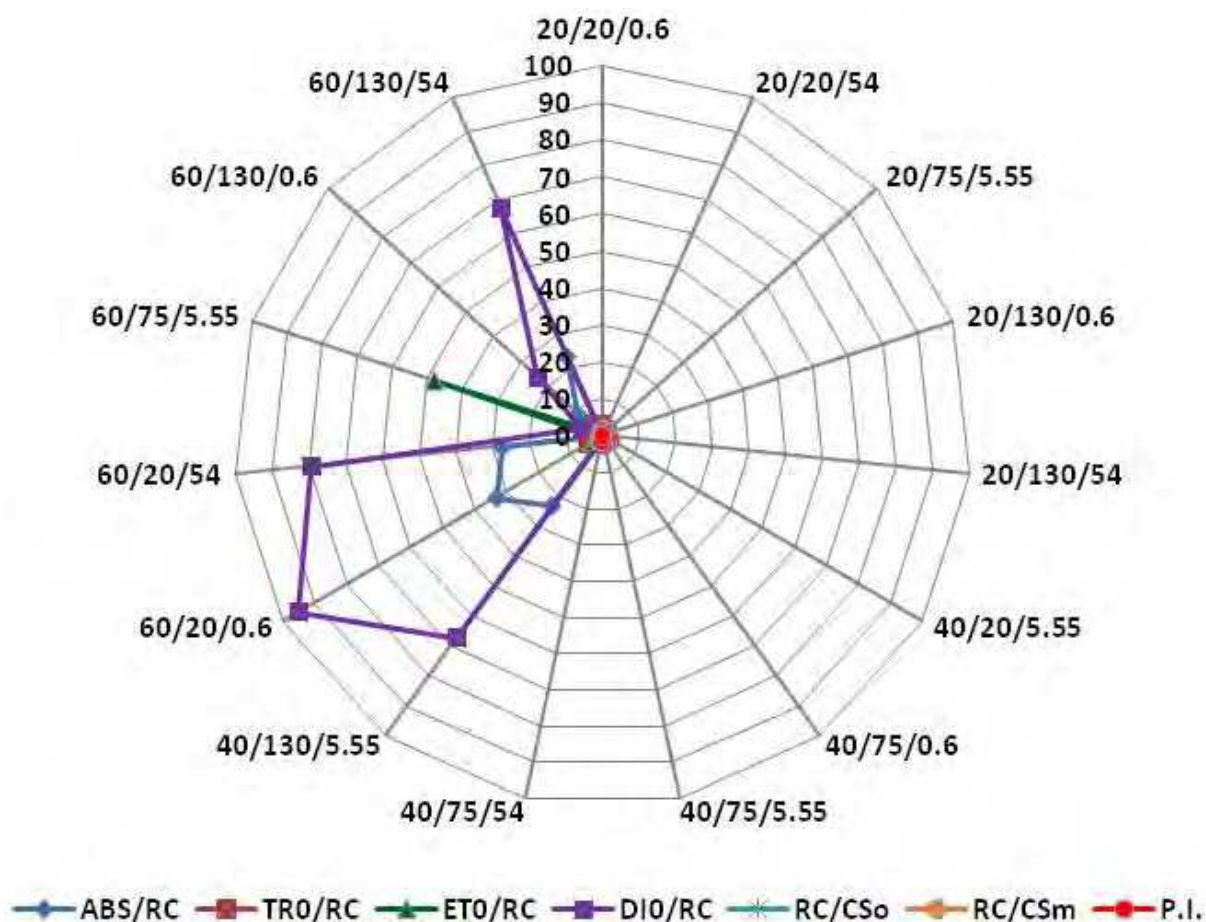

Fig. 8. Spider plot of specific energy fluxes parameters

All these results were in line with those observed by Mathur et al. (2010) and Mehta et al. (2010) in wheat leaves of *Triticum aestivum* as a response to high salt stress.

According to Zhao et al. (2008), heat stress in *Spirulina platensis* resulted in a decrease in RC/ABS, suggesting further a decrease in the total number of active reaction center. It should be pointed out that the expression RC/ABS refers to Q_A reducing reaction centers of PSII (Strasser & Strasser, 1995; Strasser et al., 2000). The decrease in RC/ABS in heat-stressed *Spirulina* cells also indicates a decrease in the total number of Q_A reducing reaction centers of PSII. These results further indicate that heat stress caused a shift of the equilibrium towards Q_A, making the electrons tend to stay longer on Q_A, suggesting that heat stress induced an increase in the Q_B non- reducing reaction centers of PSII.

Dissipation in this context refers to the loss of absorbed energy through heat, fluorescence and energy transfer to other systems (Strasser et al., 2000), and is represented by the equation $DI_0/RC= (ABS/RC)-(TR_0/RC)$. Therefore, dissipation can be thought of as the absorption of photons in excess of what can be trapped by the reaction center. In illuminated *Monstera* leaves, the excitation energy in the antenna of the active reaction centers was in excess of that required for trapping and some energy was dissipated.

The energy pipeline model as an effect of elevated temperature was deduced using biolyzer HP3 software. This model gives information about the efficiency of flow of energy from antennae to the electron transport chain components through the RC of PSII. The area or the width of the arrows for each of the parameters, ABS/CS_0, TR_0/CS_0, ET_0/CS_0 and DI_0/CS_0, indicate the efficiency of light absorption, trapping, electron transport and dissipation per cross-section of PSII, respectively, in *Cosmarium sp.* treated in 20°C and 60°C.

The effect of elevated temperature on *Cosmarium* cells was represented phenomenologically per excited cross-section (CS) area (Fig. 9).
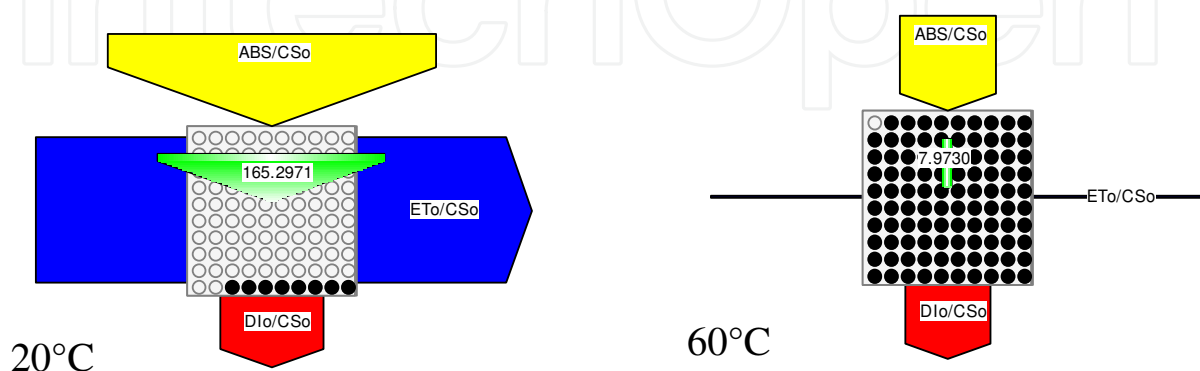

Fig. 9. Phenomenological fluxes of cell suspension pipeline model of *Cosmarium* sp. treated at two temperature conditions (20°C and 60°C).

Results showed a decrease in ABS/CS_0, ET_0/CS_0 under elevated temperature (60°C). Indeed, ABS/CS_0 describes the number of photons absorbed by antenna molecules of active and inactive PSII RCs over the excited cross-section of the tested sample and is represented by the dark-adapted F_0. The ABS/CS_0 can be substituted as an approximation by the fluorescence intensity, F_0. A decrease in ABS/CS_0 at high temperature indicates a decrease in the energy absorbed per excited cross-section. ET_0/CS_0 represents electron transport in a PSII cross-section and indicates the rate of reoxidation of reduced Q_A via electron transport over a cross-section of active RCs (Force et al., 2003). A decrease in this ratio indicates inactivation of RC complexes and the Oxygen Evolving Complex (OEC) and also suggests that the donor side of PSII has been affected.

DI_0/CS_0, represents the total dissipation measured over the cross-section of the sample that contains active and inactive RCs. A decrease in the density of active RCs (indicated as open circles) and an increase in the density of inactive RCs (indicated as filled circles) was observed in response to elevated temperature. Dissipation occurs as heat, fluorescence and energy transfer to other systems. An increase in energy dissipation at high temperature indicates that energy available for photochemistry is reduced under stress conditions (Strasser, 1987; Strasser et al., 1996, 2000; Kruger et al., 1997; Force et al., 2003).

5. Conclusion

In conclusion, our findings showed that morphological change of *Arthrospira platensis* trichome from helicoidal to the straight morphone was accompanied with physiological modifications as response to salt stress. Indeed, the increase of NaCl concentration in growth media to 500mM enhances the growth, the photosynthetic efficiency and PSII

activity as compared to 17mM cultures. The elevated PSII activity might be attributed to the increase of phycobilin content which led to an increase of light absorption by phycobilisomes relative to that of chlorophyll-a and therefore an increase of the energy transfer between phycobilisomes and PSII. Furthermore, the fluorescence emission spectra of phycobilin excitation showed that the energy in excess might be directly dissipated by fluorescence of phycobilin in order to protect the PSII against the photo inhibition.

The straight form seems to be more tolerant to salt; it maintains full activity under 500 mM NaCl medium whereas the optimal growth medium for the helicoidal morphone is 17 mM NaCl. Therefore, a better understanding of salt stress on PSII may help optimize the productivity of the microalgal cultures grown outdoors.

In order to increase and optimize the biomass productivity, this strain was performed under two trophic modes (autotrophic and mixotrophic) using factorial design. As revealed by the kinetics of maximal net photosynthetic, dark respiration rates and instantaneous growth rates, mixotrophic cultures showed two phases. The first was distinguished by the preponderance of the photoautotrophic mode while the second was based mainly on photoheterotrophy. The synergistic effect of photosynthesis and glucose oxidation enhanced the growth rate and the biomass concentration of *Arthrospira platensis* under mixotrophic mode as compared to autotrophic mode.

The combined effect of temperature, light intensity and C/N ratio on *Cosmarium sp* demonstrate that *Cosmarium* cells isolated from hot spring water maintained their maximum photochemical efficiency under 20°C. Nevertheless this specie was acclimated and withstood under 60°C by down-regulating electron transport at both donor and acceptor sides of PSII. Otherwise, the conversion of some active reaction centers of PSII into inactive form provides a protective mechanism for quenching excessive energy. Indeed the excitation energy in excess would be dissipated as heat and fluorescence or transferred to other systems via electron transport pathways such respiration or state transition between PSII and PSI suggested to be a part of the acclimation mechanisms to environmental stress.

Ultimately to withstand and grow under harsh environments or extreme conditions, several adaptive mechanisms might be developed. These mechanisms were accompanied with their ability to adjust their photosynthetic apparatus in order to acclimate to the prevailing environmental conditions.

6. References

Adams, WW., Dommig Adams, B; Rosenstiel, TN; Ebbert ,V; Brightwell, AK; Barker, DH., Zarter, C. (2001). Photosynthesis, xanthophylls, and D1 phosphorylation under winter stress., In: PS2001 *Proceedings: 12th International congress on photosynthesis.* CSIRO publishing, Melbourne, Australia.

Allakhverdiev, S.I., Nishiyama, Y., Miyairi, S., Yamamoto, H., Inagaki, N., Kanesaki ,Y., Murata, N. (2002). Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in *Synechocystis. Plant Physiol,* 130: 1443-1453.

Allen, D. J. & Ort, DR. (2001). Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Sci,* 6: 36– 42.

Andrade, MR & Costa, JAV. (2007). Mixotrophic cultivation of microalga *Spirulina platensis* apparatus, *Biochim. Biophys. Acta,* 601: 433–442.

Armond, P.A., Bjorkman, O., Staehelin, L.A. (1980). Dissociation of supramolecular complexes in chloroplast membranes. A manifestation of heat damage to the photosynthetic apparatus. *Biochim Biophys Acta,* 601 (3):433-43.

Ban Dar, H. & Leu, K.L. (2003). A possible origin of the middle phase of polyphasic chlorophyll fluorescence transient. Funct. *Plant Biol.* 30:571–6.

Ben Dhiab, R., Ben Ouada, H., Boussetta, H., Frank, F., El Abed, A., Brouers, M. (2007). Growth, Fluorescence, Photosynthetic O_2 production and pigment content of salt adapted cultures of *Arthrospira platensis*. *J Appl Phycol.* 19: 293-30.

Ben Dhiab, R., Ghenim, N., Trabelsi, L., Yahia, A., Challouf, R., Ghozzi, K., Ammar, J., Omrane, H., Ben Ouada, H. (2011). Modeling growth and photosynthetic response in *Arthrospira platensis* as function of light intensity and glucose concentration using factorial design. *Journal of Applied Phycology,* 22 (6): 745-752

Berry, S., Bolychevtseva, YV., Rögner, M., Karapetyan, NV. (2003). Photosynthetic and respiratory electron transport in the alkaliphilic cyanobacterium *Arthrospira (Spirulina) platensis. Photosynth Res* 78:67–76

Björkman, O. & Demming, B. (1987). Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* 170: 489–504

Buchel, C. & Wilhem, C. (1993). In vivo analysis of slow chlorophyll fluorescence induction kinetics in algae: progress, problems and perspectives. *Photochem Photobiol* 58: 137–148

Bukhov, N.G. & Carpentier, R. (2000). Heterogeneity of photosystem II reaction centers as influenced by heat treatment of barley leaves. *Physiologia Plantarum*, 110: 279–285

Cajanek, M., Stroch, M., Lachetova, I., Kalina, J., Spunda, Y. (1998). Characterization of the photosystem II inactivation of heat stressed barley leaves as monitored by the various parameters of chlorophyll a fluorescence and delayed fluorescence. Journal of *Photochemistry and Photobiology*, 47, 39–45

Campbell, D., Hurry, V., Clarke, A., Gustafson, P., Oquist, G. (1998). Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol Mol Biol Rev,* 62: 667–683

Chen, F. & Zhang, Y. (1997). High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. *Enzyme Microb Technol*, 20: 221-224

Chen, F., Zhang, Y., Guo, S. (1996). Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. *Biotechnol Lett*, 18: 603-608

Chojnacka, K. & Marquez-Rocha, FJ. (2004.) Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnology*, 3: 21-34

Chojnacka, K. & Noworyta, A. (2004). Evaluation of *Spirulina sp.* Growth in photoautotrophic, heterotrophic and mixotrophic cultures. *Enzyme Microb Technol*, 34: 461-465

Chojnacka, K. (2003). Heavy metal ions removal by microalgae *Spirulina sp.* In the processes of biosorption and bioaccumulation. Dissertation, Wroclaw University of Technology Poland.

Endo, T., Schreiber, U., Asada, K. (1995). Suppression of quantum yield of photosyntem II by hyperosmotic stress in *Chlamydomonas reinhardtii, Plant Cell Physiol.* 36: 1253–1258

Fabregas, J., Herrero, C., Cabezas, B., Liano, R., Abalde, J. (1986). Response of the marine microalga *Dunaliella tertiolecta* to nutrient concentration and salinity variations in batch cultures. *Journal of Plant Physiology,* 125: 475-4-84

Falkowski, PG. & LaRoche, J. (1991). Acclimation to spectral irradiance in algae. *Journal of Phycology*, 27:8-14

Force, L., Gritchley, C., Van Rensen, JJS. (2003). New fluorescence parameters for monitoring photosynthesis in plants 1. The effect of illumination on the fluorescence parameters of the JIP test. *Photosynth Res*, 78: 17-33

Geider, RJ. (1987). Light and temperature dependence of carbon to chlorophyll a ratio in microalgae and cyanobacteria: implications of physiology and growth of phytoplankton. *New Phycologist*, 106: 1-34

Georgieva K., Tsonev T., Velikova V., Yordanov I. (2000). Photosynthetic activity during high temperature of pea plants. *Journal of Plant Physiology*, 157: 169–176

Giardi, MT., Masojidek, J., Godde, D. (1997). Effects of abiotic stresses on the turnover of the D1 reaction center II protein. *Physiol. Plant*. 101, 635-642

Gilmour, DJ., Hipkins, MF., Boney, AD. (1982). The effect of salt stress on the primary processes of photosynthesis in *Dunalielia tertiolecta. Plant Sci. Lett.* 26, 325-330

Gilmour, DJ., Hipkins, MF., Webber, AN., Baker, NR., Boney, AD. (1985). The effect of ionic stress on photosynthesis in *Dunaliella tertiolecta, Planta* 163: 250–256

Goncalves, JFC. & Santos, UM. (2005). Fluorescence technique as a tool for selecting tolerant species to environments of high irradiance. *Brazil. J. Plant Physiol,* 17: 307–13

Govindjee (1995). Sixty-three years since Kautsky: chlorophyll a fluorescence. Austr. J. Plant Physiol. 22, 131–160

Govindjee (2004). Chlorophyll a fluorescence: a bit of basics and history. In Papageorgiou,

GC. & Govindjee (eds.) Chlorophyll a fluorescence: A signature of photosynthesis. Springer, Dordrecht, the Netherlands, pp. 321-362.

Hase, R., Oikaw,a O., Sasao, C., Morita, M., Watanabe, Y. (2000). Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai city. *J Biosci Bioeng*, 89: 157-163

Hata, J.I, Hua, Q., Yang, C., Shimizu, K., Taya, M. (2000). Characterization of energy conversion based on metabolic flux analysis in mixotrophic liverwort cells, *Marchantia polymorpha. Biochem Eng J*, 6: 65-74

Havaux, M. (1993). Characterization of thermal damage to the photosynthetic electron transport system in potato leaves, *Plant Sci*, 94: 19–33

Hermans, C., Smeyers, M., Rodriguez, RM., Eyletters, M., Strasser, RJ. & Delhaye, JP. (2003). Quality assessment of urban trees: a comparative study of physiological characterization, airborne imaging and on site fluorescence monitoring by the OJIP-test. *J. Plant Physiol,* 160: 81–90.

Hill, R. & Ralph, PJ. (2005). Diel and seasonal changes in fluorescence rise kinetics of three scleractinian corals. *Funct. Plant Biol*. 32: 549–59

Hill, R., Larkum, AWD., Frankart, C., Kuhl, M., Ralph, PJ. (2004). Loss of functional photosystem II reaction centers in zooxanthellae of corals exposed to bleaching conditions: using fluorescence rise kinetics. *Photosynth. Res,* 82: 59–7.

Huner, NPA., Oquist, G., Sarhan, F. (1998). Energy balance and acclimation to light and cold. *Trends Plant Sci*, 3: 224–30

Jeeji Bai, N. (1985). Competitive exclusion or morphological transformation? A case study with *Spirulina fusiformis*. *Arch Hydrobiol Suppl* 71, *Algal Stud*, 191: 38–39

Joset, F., Jeanjean, R., Hagemann, M. (1996). Dynamics of the response of cyanobacteria to salt stress: deciphering the molecular events. *Physiol. Plant,* 96: 738-744

Kalaji, HM., Govindjee, Bosa, K., Koscielniak, J., Golaszewska, KZ. (2011). Effects of salt stress on photosystem II efficiency and CO₂ assimilation of two Syrian barley landraces. Environmental and Experimental Botany 73: 64-72

Kana, T., Gilbert, PM. (1987). Effect on irradiance up to 2000 µE m⁻²s⁻² on marine *Synechococcus* WH 7803-I. Growth: pigmentation and cell composition. *Deep-Sea Res,* 34: 479–516

Kana, TM., Feiwel, NL., Flynn, LC. (1992). Nitrogen starvation in marine *Synechococcus* strains: clonal differences in phycobiliprotein breakdown and energy coupling. *Marine Ecology Progress Series,* 88: 75-82.

Kang, R., Wang, J., Shi, D., Cong, W., Cai, Z., Ouyang, F. (2004). Interaction between organic and inorganic carbon sources during mixotrophic cultivation of *Synechococcus sp. Biotechnol Lett,* 26: 1429-1432

Kirst, GO. (1990). Salinity tolerance of eukaryotic marine algae, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41: 21–53.

Kramer, DM., Avenson, TJ., Kanazawa, A., Cruz, JA., Ivanov, B., Edwards, GE. (2004). Therelationship between photosynthetic electron transport and its regulation. In Papageorgiou, GC. & Govindjee (eds.) Chlorophyll a Fluorescence: A Signature of Photosynthesis. Springer, Dordrecht, the Netherlands, pp. 251–78.

Krauss, N., Hinrichs, W., Witt, I., Fromme, P., Protzkow, W. (1993). 3-Dimensional structure of the system-I of photosynthesis at 6 angstrom resolution. *Nature,* 361: 326-331

Krüger, GHJ., Tsimilli-Michael, M., Strasser, RJ. (1997). Light stress provokes plastic and elastic modifications in structure and function of photo system II in camellia leaves. *Physiologia Plantarum*, 101: 265-277

Kruskopf, M. & Flynn, KJ. (2006). Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. *New Phytol,* 169: 525–36

Laws, EA. & Bannister, (1980). Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture with implications for phytoplankton growth in the ocean. *Limnol. Oceanog*, 25 (3): 457-473

Lazar, D. (2006). The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. *Funct. Plant Biol,* 33: 9-30

Lewin, RA. (1980.) Uncoiled variants of *Spirulina platensis* (Cyanophyceae: Oscillatoriaceae). *Arch Hydrobiol* Suppl 60, *Algal Stud,* 26: 48–52

Li, WKW., Smith, JC., Platt, T. (1984). Temperature response of photosynthetic capacity and carboxylase activity in Arctic marine phytoplankton. *Marine Ecology Progress Series*, 17: 237-243.

Liu, X., Duan, S., Li, A., Xu, N., Cai, Z., Hu, Z. (2009). Effect of organic carbon sources on growth, photosynthesis, and respiration of *Phaeodactylum tricornutum. J Appl Phycol*, 21: 239-246

Lodi, A., Binaghi, L., De Faveri, D., Carvalho, JCM., Converti, A. (2005). Fed-batchmixotrophic cultivation of *Arthrospira (Spirulina) platensis* (Cyanophycea) with carbon source pulse feeding. *Ann Microbiol*, 55: 181-185

Lu, C. & Zhang, J. (1999). Effects of salt stress on PSII function and photoinhibition in cyanobacterium *Spirulina platensis, J. Plant Physiol*, 155: 740–745.

Lu, C. & Zhang, J. (2000). Role of light in the response of PSII photochemistry in the cyanobacterial *Spirulina platensis* to salt stress, *J. Exp. Bot,* 51: 911–917.

Lu, CM., Torzillo, G., Vonshak, A. (1999). Kinetic response of photosystem II photochemistry in cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases. *Aust J Plant Physiol,* 26: 283–292

Lu, CM. & Vonshak, A. (1999). Characterization of PSII photochemistry in salt adapted cells of cyanobacterium *Spirulina platensis. New Phytol*, 141: 231–239

Lu, CM. & Vonshak, A. (2002). Effect of salinity on photo system II function in cyanobacterial *Spirulina platensis* cells. *Physiol Plant,* 114: 405–413

Lu, CM., Zhang, J., Vonshak, A. (1998). Inhibition of quantum yield of PSII electron transport *in Spirulina platensis* by osmotic stress may be explained mainly by an increase in the proportion of the Q_B non reducing PSII reaction centres. *Aust J Plant Physiol*, 25: 689–694

Marquez, FJ., Nishio, N., Nagai, S., Sasaki, K. (1995). Enhancement of biomass and pigment production during growth of *Spirulina platensis* mixotrophic culture. *J Chem Tech Biotech*, 62: 159-164

Marquez, FJ., Sasaki, K., Kakizono, T., Nishio, N., Nagai, S. (1993). Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. *J Ferment Bioeng,* 76: 408-410

Martinez, F. & Orus, MI. (1991). Interactions between glucose and inorganic carbon metabolism in *Chlorella vulgaris* strain UAM 101. *Plant Physiol*, 95: 1150-1155

Martinez, F., Camacho, F., Jiménez, JM., Espinola, JB. (1997). Influence of light intensity on the kinetic and yield parameters of *Chlorella pyrenoidosa* mixotrophic growth. *Process Biochem*, 32: 93-98

Mathur, S., Jajoo, A., Mehta, P., Bharti, S. (2010). Analysis of elevated temperature-induced inhibition of Photosystem II by using chlorophyll a fluorescence induction kinetics in wheat leaves (*Triticum astivum*). *Plant Biol,* doi:1111/j-1438-8677-2009.00319.X

Maxwell, K. & Johnson, GN. (2000). Chlorophyll fluorescence- a practical guide. *J Exp. Bot.* 51: 659–68

Mehta, P., Jajoo, A., Mathur, S., Bharti, S. (2010). Chlorophyll a fluorescence study effects of high salt stress on photosystem II in wheat leaves. *Plant Physiol. Biochem,* 48: 16-20

Melis, A. (1999). Photosystem II damage and repair cycle in chloroplasts : what modulates the rate of photodamage in vivo ? *Trends Plant Sci,* 4: 130-135

Nash, D., Miyao, M., Murata, N. (1985). Heat inactivation of oxygen evolution in photosystem II particles and its acceleration by chloride depletion and exogenous manganese, *Biochim. Biophys. Acta,* 807 127–133

Neubauer, C. & Schreiber, U. (1987). The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination: 1. Saturation characteristics and partial control by the Photo system II acceptor side. *Z Naturforsch*, C 42: 1246-1254

Orus, MI., Marco, E., Martinez, F. (1991). Suitability of *Chlorella vulgaris* UAM 101 for heterotrophic biomass production. *Bioresour Technol*, 38: 179-184

Osborne, BA. & Geider, RJ. (1986). Effect of nitrate limitation on photosynthesis of the diatom *Phaeodactylum tricornutum Bohlin* (Bacillarophyceae). *Plant, Cell and Environment* 9: 617-625

Papageorgiou, G. & Govindjee (1968). Light induced changes in the fluorescence yield of chlorophyll a in vivo *Anacystis nidulans. Biophys J*, 8: 1299–1315

Papageorgiou, GC. (1996). The photosynthesis of cyanobacteria (blue bacteria) from the perspective of signal analysis of chlorophyll a fluorescence. *J Sci Ind Res* 55: 596–617

Pelroy, RA., Rippka, R., Stanier, RY. (1972). Metabolism of glucose by unicellular blue-green algae. *Archiv für Mikrobiologie,* 87: 303-322

Pereira, WE., de Siqueira, DI., Martinez, CA., Puiatti, M. (2000). Gas exchange andchlorophyll fluorescence in four citrus rootstocks under aluminium stress. *J. Plant Physiol,* 157: 513-520

Pogoryelov, D., Sudhir, PR., Kovàcs, L., Combos, Z., Brown, I., Garab, G. (2003). Sodium dependency of the photosynthetic electron transport in the alkaliphilic cyanobacterium *Arthrospira platensis. J Bioenerg Biomembranes*, 35: 427–437

Pospisil, P. & Tyystjarvi, E. (1999). Molecular mechanism of high-temperature-induced inhibition of acceptor side of photosystem II, *Photosynth. Res,* 62: 55–66

Powles, SB. (1984). Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant Physiol,* 35: 15–44

Prasil, O., Adir, N., Ohad, I. (1992). Dynamics of photosystem II: mechanism of photoinhibition and recovery process. *Topics in photosynthesis,* 11: 295-348

Qiu, B., Zhang, A., Zhou, W., Wei, J., Dong, H., Liu, Z. (2004). Effects of potassium on the photosynthetic recovery of the terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae) during rehydration. *J. Phycol,* 40: 323–32

Raven , JA. & Geider, RJ. (1988). Temperature and algal growth. *New Phycologist,* 110: 441-461.

Rhee, GY. & Gotham, IJ. (1981). The effect of environmental factors on phytoplankton growth : light and the interactions of light with nitrate limitation. *Limnol. Oceanogr*, 26 (4): 649-659

Richardson, K., Beardall, J., Raven, JA. (1983). Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytologist,* 93: 157-191

Richmond, A. (1988). *Spirulina*, In: Borowitzka, MA., Borowitzka, LJ. (eds.) Microalga biotechnology. Cambridge, Cambrige University Press, pp 85-121

Schreiber, U. & Bilger, W. (1987). Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. In: Tenhunen GD. (eds.), Plant response to stress. Springer Verlag, New York, pp. 27-53

Schreiber, U. & Neubauer, C. (1987). The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination. 2. Partial control by the photosystem II donor side and possible ways of interpretation. *Z. Naturforsch,* C 42: 1255-1264

Schreiber, U., Bilger, W., Neubauer, C. (1994). Chlorophyll fluorescence as a non invasive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, ED., Caldwell, MM. (eds.) Ecophysiology of photosynthesis. Springer, Berlin Heidelberg New York, pp 49-70

Schreiber, U., Schliwa, U., Bilger, W. (1986). Continuous recording of photochemical and non photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth Res,* 10: 51–62

Schubert, H., Fluda, S., Hagemann, M. (1993). Effects of adaptation to different salt concentrations on photosynthesis and pigmentation of the cyanobacterium *Synechocystis sp.* PCC 6803. *J. Plant Physiol,* 142 (3): 291-295

Schubert, H. & Hagemann, M. (1990). Salt effects on 77K fluorescence and photosynthesis in the cyanobacterium *Synechocystis sp.* PCC 6803, *FEMS Microbiol. Lett*, 71: 169–172.

Srivastava, A., Strasser, RJ., Govindjee (1999). Greening of peas: parallel measurements of 77K emission spectra, OJIP chlorophyll a fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. *Photosynthetica,* 37: 365-392

Strasser, BJ. (1997). Donor side capacity of PSII probed by chlorophyll a fluorecence transients. *Photosynth, Res*, 52: 147-155

Strasser, BJ., Eggenberg, P., Strasser, BJ. (1996). How to work without stress but with fluorescence. *Bulletin de la Societé Royale des Sciences de Liege*, 65: 330-349

Strasser, BJ. & Strasser, RJ. (1995). Measuring fast fluorescence transients to address environmental questions: the JIP test. In: Mathis, P. (eds.) Photosynthesis: from light to biosphere. Kluwer, Dordrecht, pp 977-980

Strasser, RJ. (1987). Energy pipeline model of the photosynthetic apparatus. In: Biggins, J. (eds.), Progress in Photosynthesis Research. Matinus Nijhoff Publishers, Dordrecht, The Netherlands, pp 717–720

Strasser, RJ., Srivastava, A., Govindjee (1995). Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochem Photobiol*, 61: 32-42

Strasser, RJ., Srivastava, A., Tsimilli-Michael, M. (2000). The fluorecence transient as a tool to characterize and screen photosynthetic samples. In Yunus, M., Pathre, U., Mohanty, P. (eds.), Probing Photosynthesis Mechanisms, regulation and Adaptation. Taylor and Francis, London, UK, pp 445-483.

Strasser, RJ., Tsimilli-Michael, M., Srivastava, A. (2004). Analysis of the chlorophyll a fluorescence transient. In Papageorgiou, GC. & Govindjee (eds.) Chlorophyll a Fluorescence: A Signature of Photosynthesis. Springer, Dordrecht, the Netherlands, pp. 321–62.

Tomek, P., Lazar, D., Ilik, P., Naus, J. (2001). On the intermediate steps between the O and P steps in chlorophyll a fluorescence rise measured at different intensities of exciting light. *Aust J Plant Physiol*, 28: 1151–1160

Torzillo, G., Bernardini, P., Masojidek, J. (1998). On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina plantensis* (cyanobacteria). *J. Phycol*, 34: 504–10

Toth, SZ. & Strasser, RJ. (2005). The specific rate of QA reduction and photosystem II heterogeneity. In: Van Est, A. & Bruce, D. (eds.) Photosynthesis: fundamental aspects to global perspectives. Allen Press Inc, Lawrence, pp 198–200

Toth, SZ., Schansker, G., Garab, G., Strasser, R.J. (2007). Photosynthetic electron transport activity in heat-treated barley leaves: the role of internal alternative electron donors to photosystem II, *Biochim. Biophys. Acta*, 1767: 295–305

Tsonev, T., Velikova, V., Georgieva, K., Hyde, PF., Jones, HG. (2003). Low temperature enhances photosynthetic downregulation in French bean (*Phaseolus vulgaris* L.) plants. *Ann. Bot*, 91:343–52.

Tsonev, TD. & Hikosaka, K. (2003). Contribution of photosynthetic electron transport, heat dissipation, and recovery of photoinactivated photosystem II to photoprotection at different temperatures in *Chenopodium album* leaves. *Plant Cell Physiol*, 44: 828-835

Verity, PG. (1982). Effects of temperature, irradiance and daylength on the marine diatom *Leptocylindrus danicus* Cleve. 4. Growth. *Journal of Experimental Marine Biology and Ecology* 60: 209-222

Vonshak, A. & Novoplansky, N. (2008). Acclimation to low temperature of two *Arthrospiraplatensis* (Cyanobacteria) strains involves down regulation of PSII and improved resistance to photoinhibition. *J. Phycol*, 44: 1071–1079

Vonshak, A., Chanawonge, L., Bunnag, B., Tanticharoen, M. (1995). Physiological characterization of *Spirulina platensis* isolates: Response to light and salinity. *Plant Physiol,* 14: 161–166

Vonshak, A., Cheung, SM., Chen, F. (2000). Mixotrophic growth modifies the response of *Spirulina (Arthrospira) platensis* (cyanobacteria) cells to light. *J Phycol,* 36: 675-679

Vonshak, A., Guy, R., Guy, M. (1988). The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. *Arch Microbiol*, 150: 417–420

Vonshak, A., Kancharaksa, N., Bunnag, B., Tanticharoen, M. (1996). Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. *J Appl Phycol*, 8: 19–124

Wen, XG., Gong, HM., Lu, C. (2005). Heat stress induces an inhibition of excitation energy transfer from phycobilisomes to photosystem II but not to photosystem I in a cyanobacterium *Spirulina platensis, Plant Physiol. Biochem*, 43: 389–395.

Wilson, KE., Ivanov, AG., Oquist, G., Grodzinski, B., Sarhan, F., Huner, NPA. (2006). Energy balance, organellar redox status, and acclimation to environmental stress. *Can. J. Bot,* 84: 1355-1370

Yamamoto, Y. (2001). Quality control of photosystem II. *Plant Cell Physiol*, 42: 121-128

Yamane, Y., Kashino, Y., Koike H., Satoh, K. (1998). Effects of high temperature on the photosynthetic systems in spinach oxygen-evolving activities, fluorescence characteristics and the denaturation process, *Photosynth. Res*, 57: 51–59

Yang, C., Hua, Q., Shimizu, K. (2000). Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions. *Biochem Eng J*, 6: 87-102

Young, AJ. (1993). Factors that affect the carotenoid composition of higher plants and algae. In Carotenoids in Photosynthesis, Young, AJ. & Britton, G. (eds.) London: Chapman and Hall, pp. 161-205.

Yu, H., Jia, S., Dai, Y. (2009). Growth characteristics of the cyanobacterium Nostoc flagelliforme in photoautotrophic, mixotrophic and heterotrophic cultivation. *J Appl Phycol,* 21: 127-133

Zeng, MT. & Vonshak, A. (1998). Adaptation of *Spirulina platensis* to salinity stress. *Comp Biochem Physiol*, Part A 120: 113-118

Zhang, D., Pan, X., Mu, G., Wang, J. (2010). Toxic effects of aantimony on photosystem II of *Synechocystis sp.* As probed by in vivo chlorophyll fluorescence. *J. Appl Phycol,* 22: 479-488

Zhang, XW., Zhang, YM., Chen, F. (1999). Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis. Process Biochem,* 34: 477-481

Zhao, B., Wang, J., Gong, H., Wen, X., Ren, H., Lu, C. (2008). Effects of heat stress on PSII photochemistry in a cyanobacterium *Spirulina platensis*. *Plant Science*, 175: 556-564

Zhu, XG., Govindjee, Baker, NR., DeSturler, E., Ort, DR., Long, SP. (2005). Chlorophyll a fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with photosystem II. *Planta*, 223: 114-133.
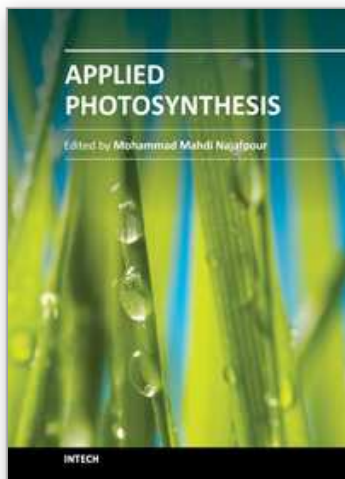
Applied Photosynthesis

Edited by Dr Mohammad Najafpour

Photosynthesis is one of the most important reactions on Earth, and it is a scientific field that is intrinsically interdisciplinary, with many research groups examining it. This book is aimed at providing applied aspects of photosynthesis. Different research groups have collected their valuable results from the study of this interesting process. In this book, there are two sections: Fundamental and Applied aspects. All sections have been written by experts in their fields. The book chapters present different and new subjects, from photosynthetic inhibitors, to interaction between flowering initiation and photosynthesis.

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