J. Phycol. **36,** 675–679 (2000)

MIXOTROPHIC GROWTH MODIFIES THE RESPONSE OF *SPIRULINA (ARTHROSPIRA) PLATENSIS* (CYANOBACTERIA) CELLS TO LIGHT[1]

Avigad Vonshak[2]

Laboratory for Microalgal Biotechnology, The Albert Katz Department of Dryland Biotechnologies, Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede-Boker Campus 84990, Israel

Suk Man Cheung and Feng Chen

Department of Botany, The University of Hong Kong, Pokfulam Road, Hong Kong

***Spirulina (Arthrospira) platensis* (Nordstedt) Geitler cells grown under mixotrophic conditions exhibit a modified response to light. The maximal photosynthetic rate and the light saturation value of mixotrophic cultures were higher than those of the photoautotrophic cultures. Dark respiration and light compensation point were also significantly higher in the mixotrophically grown cells. As expected, the mixotrophic cultures grew faster and achieved a higher biomass concentration than the photoautotrophic cultures. In contrast, the growth rate of the photoautotrophic cultures was more sensitive to light. The differences between the two cultures were also apparent in their responses to exposure to high photon flux density of 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The light-dependent O_2 evolution rate and the maximal efficiency of photosystem II photochemistry declined more rapidly in photoautotrophically grown than in mixotrophically grown cells as a result of exposure to high photon flux density. Although both cultures recovered from the high photon flux density stress, the mixotrophic culture recovered faster and to a higher extent. Based on the above results, growth of *S. platensis* with a fixed carbon source has a significant effect on photosynthetic activity.**

Key index words: **growth rate; mixotrophic; photoinhibition; photosynthesis; PS II; *Spirulina (Arthrospira) platensis***

Abbreviations: **E_c, light compensation point; E_k, light saturation value; HPFD, high photon flux density; P_{max}, maximal photosynthetic rate; R_d, dark respiration rate**

During mixotrophic growth of algae, both light and fixed carbon are used as a source of energy. Much work has been done on heterotrophic and mixotrophic growth of the green algae *Chlorella* (Endo et al. 1977, Guminski et al. 1985, Misonou and Pachlavuni 1986), *Scenedesmus* (Shamala et al. 1982), and *Haematococcus* (Kobayashi et al. 1992). These algae can use different organic carbon sources, such as glucose and acetate. Although a number of unicellular cyanobacteria can use various sources of fixed carbon (Rippka 1972), studies on mixotrophic and heterotrophic growth of cyanobacteria are limited.

Commercial mass cultivation of *Spirulina platensis* as a food supplement has existed since the late 1970s (Ciferri 1983, Vonshak 1997). Cultivation is performed mainly in open shallow ponds so that solar energy absorbed by the cyanobacteria is used to fix inorganic carbon. This mode of growth results in a relatively low biomass concentration, which requires large cultivation systems. Culturing *S. platensis* in mixotrophic conditions could potentially yield a higher biomass concentration (Marquez et al. 1995, Chen and Zhang 1997). With recent improvements in closed systems for mass cultivation of microalgae, mixotrophic growth of cyanobacteria may become economically feasible in the near future.

The filamentous cyanobacterium *S. platensis* was shown to be capable of using glucose as an organic carbon source (Marquez et al. 1993, Chen et al. 1996). The biomass and photosynthetic pigments produced during mixotrophic growth were increased by 1.5- to 2-fold, compared with those in photoautotrophic cultures. However, the pigment content per biomass of the mixotrophic culture was almost the same as in the photoautotrophic culture (Marquez et al. 1993). In the same work, it was reported that dark respiration and light-dependent O_2 evolution of autotrophic and mixotrophic *S. platensis* cells were not significantly different. Recently, Marquez-Rocha (1999) published a re-assessment of the bioenergetic growth yield of *Arthrospira platensis* using continuous culture under autotrophic and mixotrophic conditions, demonstrating that at any given dilution rate, the mixotrophic culture had a higher bioenergetic yield. Yet the changes in the pigment content in both cultures were better correlated to the light intensity than to the mode by which the carbon was supplied.

Based on the finding that the total growth yield of *S. platensis* mixotrophic cultures sums to the net contribution of photoautotrophic growth in the light and heterotrophic growth in the dark, Marquez et al. (1993) suggested that the photosynthetic activity and the organic carbon-dependent respiratory activity operate separately in those cells.

Morphological changes induced in *S. platensis* grown mixotrophically have been examined (Ignat′evskaya

[1] Received 1 November 1999. Accepted 23 March 2000.

[2] Author for correspondence: e-mail avigad@bgumail.bgu.ac.il.

et al. 1996), but little is known about modifications induced by mixotrophic growth on the photosynthetic activity and responses to light. This study compares the photosynthetic activities and responses to light of photoautotrophic and mixotrophic *S. platensis* cultures.

MATERIALS AND METHODS

Organism and growth conditions. *Spirulina platensis* (Nord.) Geitler, University of Texas Culture Collection (UTEX, Austin, TX) no.1926, was grown in batch culture in Zarouk's medium containing 0.2M $NaCO_2$ (Vonshak et al. 1982) with or without supplementation of 2 $g \cdot L^{-1}$ glucose. The cells were grown in 250 mL Erlenmeyer flasks containing 100 mL of nutrient medium with continuous illumination and shaking (130 rpm). The atmosphere of the incubator was enriched with 1% CO_2 in air. The pH of the cultures was monitored daily to avoid carbon limitation. Illumination was provided by cool-white fluorescent lamps.

Oxygen evolution. Cells were harvested by centrifugation and resuspended in fresh nutrient medium to a final concentration of 4.0 mg chl$\cdot L^{-1}$. Their photosynthetic activity was assayed by measuring the rate of O_2 evolution using a Clark-type O_2 electrode (in a double-jacket, thermoregulated cylindrical glass vessel). The temperature was kept constant (30° C) and illumination was provided by a slide projector lamp at a photon flux density (PFD) of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Rates were taken from the initial activity after 2 min of light adaptation.

Fluorescence measurements. Variable chlorophyll fluorescence is used for the detection of changes induced by stress conditions in the photosynthetic apparatus. The parameters used for this measurement are Fv = Fm − Fo. Fo and Fm are the minimal and maximal fluorescence yields of a dark-adapted sample, with all PS II reaction centers fully open or closed, respectively. The ratio of the variable fluorescence to maximal fluorescence (Fv/Fm) is interpreted as a measure of the maximal quantum efficiency of PS II photochemistry (for more nomenclature and definitions see Van-Kooten and Snel 1990). Fv/Fm was measured in algal samples of photoautotrophic and mixotrophic cultures diluted to a final concentration of 5.0 mg chl$\cdot L^{-1}$ and then dark-adapted for 5 min. Measurements were performed using a plant efficiency analyzer (PEA, Hansatech, Norfolk, UK) equipped with a liquid sample holder.

Photoinhibition and recovery treatments. Algal cells at the log-phase of growth were harvested by centrifugation and resuspended in fresh nutrient medium to yield a chlorophyll concentration of 25 mg$\cdot L^{-1}$. The cultures were placed in a thermoregulated, double-jacket cylindrical glass vessel and then illuminated at a HPFD of 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ by a high-intensity halogen lamp (OSRAM [München, Germany] 220−230 V, 1000 W). Li-180 photometer and a quantum sensor were used to measure the photosynthetic active radiance (PAR) photon flux density. At given intervals, samples were withdrawn and diluted with fresh medium to 4.0 and 5.0 mg chl$\cdot L^{-1}$ for determination of oxygen evolution and Fv/Fm values, respectively. For recovery from photoinhibition, the cell suspensions were diluted to a concentration of 4.0 mg chl$\cdot L^{-1}$ with fresh medium and then incubated under dim light of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At given intervals, oxygen evolution and Fv/Fm values were measured.

Light response experiments. The procedure was the same as for the oxygen evolution determination. Light intensities were varied by using neutral density filters.

Chlorophyll a concentration was determined according to the method of Bennet and Bogorad (1973). The specific growth rate (μ) was calculated from the growth curve using a fitting program for the data that was obtained during the logarithmic phase of growth, using the following formula: $\mu = (\ln X_2 - \ln X_1)/t_2 - t_1$, where X_1 and X_2 are the biomass concentration at time t_1 and t_2, respectively, and units of μ are the reciprocal of time.

RESULTS

Effect of light on growth of photoautotrophic and mixotrophic cultures. The growth pattern of *S. platensis* cells grown under photoautotrophic or mixotrophic conditions is depicted in Fig. 1. Most apparent is the effect of light intensity on the maximal biomass concentration achieved when the cells reach the stationary phase. In photoautotrophically grown cultures, the final biomass concentration increased when the light intensity was increased from 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When the light was increased to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a photoinhibitory effect was observed. In the mixotrophically grown cultures a somewhat different response was observed. Cultures grown at a light intensity between 75 and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ reached almost the same maximal biomass concentration, whereas only the culture grown at 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ reached a much lower biomass concentration at a significantly reduced growth rate.

The effect of light on the specific growth rates of photoautotrophic and mixotrophic cells in logarithmic growth was compared (Fig. 2). Mixotrophic cultures have a higher growth rate at any given light intensity (20% to 40% higher). An inhibitory effect of light on growth is observed only at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and is more pronounced in the photoautotrophic

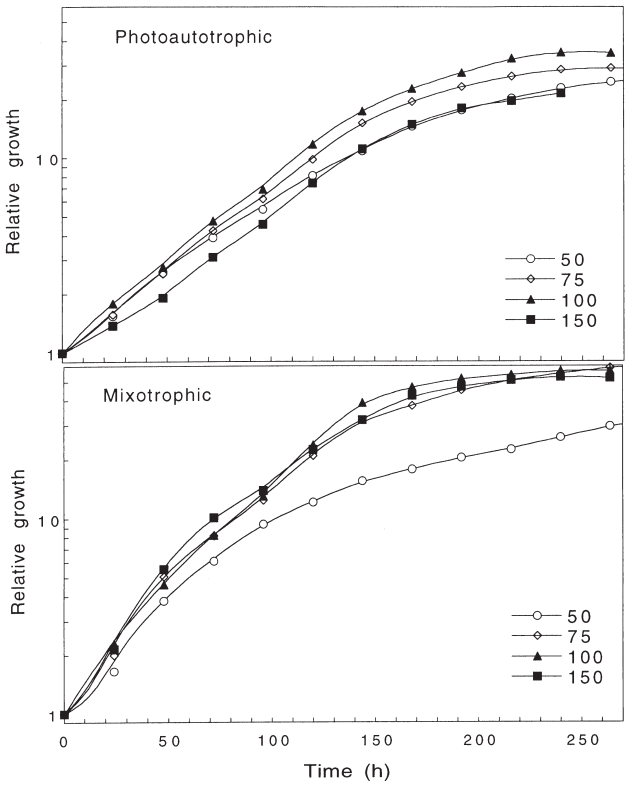

FIG. 1. Growth pattern of *Spirulina platensis* cells grown photoautotrophically or mixotrophically (50 mM glucose) under different photon flux densities. ○ 50, ◇ 75, ▲ 100, ■ 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

cells than in the mixotrophic cells (Fig. 2A). To evaluate further the response of the growth of the two cultures to light, the specific growth rate of both was measured as a function of increasing light intensity. Fig. 2B demonstrates that the growth rate of the photoautotrophic culture is more light dependent than that of the mixotrophic culture. Light levels above those that saturate growth (100−150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) can cause a marked reduction in the growth rate.

Effect of light on the photosynthetic activity of photoautotrophic and mixotrophic cultures. The different growth response to light of *S. platensis* grown under different metabolic conditions may reflect differences in photosynthetic activity. Therefore, oxygen evolution as a function of light intensity (P-I curve) of photoautotrophic and mixotrophic cultures was compared. Significant differences in the P-I curves were observed (Fig. 3, inset). The only parameter calculated from the P-I curves that did not change is the initial slope, α, which is an indication of photosynthetic efficiency (Fig. 3). All other parameters, summarized in Table 1, were significantly different. The dark respiration rate, R_d, and the light-saturated rate of photosynthesis, P_{max}, were both higher in mixotrophic than in photoautotrophic cultures. Moreover, the saturation irradiance, E_k, of the mixotrophic culture was higher than that of the photoautotrophic culture. These results suggest that although both cultures have a similar photosynthetic efficiency, they differ in their photosynthetic capacity.

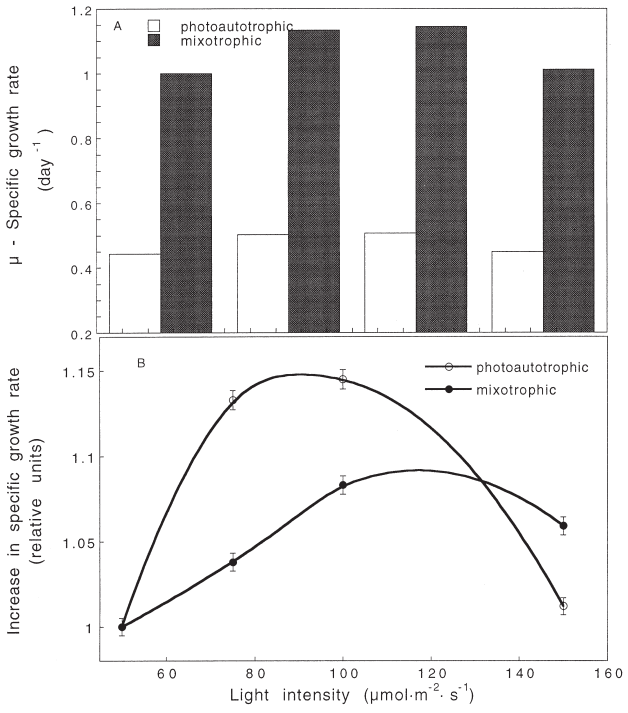

FIG. 2. Effect of light intensity on the maximal biomass concentration (A) and the relative increase in growth rate (B) in photoautotrophic and mixotrophic *Spirulina platensis* cultures.

Photoinhibition and recovery in photoautotrophic and mixotrophic cultures. To test whether the difference in the photosynthetic capacity of cells grown photoautotrophically and mixotrophically results in a modified response to light stress, the two cultures were exposed to HPFD and oxygen evolution and Fv/Fm were monitored. The decline in maximal photochemical efficiency of PS II (Fv/Fm) and the light-dependent O_2 evolution after exposure of the cultures to HPFD (3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) is shown in Fig. 4. The rate and the extent of inhibition in the mixotrophic cultures were reduced relative to the photoautotrophic cultures.

To compare the recovery of both cultures after exposure to HPFD for 30 min, the cells were placed in dim light (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and the increase in the light-dependent O_2 evolution rate was measured. As shown in Fig. 5, the photoinhibited mixotrophic cells recovered faster and to a higher extent than the photoinhibited photoautotrophic cells recovered.

DISCUSSION

Photoautotrophic microalgae use light as the sole energy source that is used for splitting molecules of water. The energy produced in this reaction is used for fixation (reduction) of CO_2, whereas under heterotrophic conditions, the requirement for light as the source of energy is eliminated and the energy required for growth is supplied via respiration, using organic carbon as the substrate. At these two extremes, different sources of energy are used, operating under distinctly different metabolic processes, whereas in mixotrophic conditions both processes are operating simultaneously.

Our data suggest that mixotrophic cultures require less light for growth. Thus, growth yield on the basis of light use is higher in mixotrophic cultures. In addition, in mixotrophic cultures, the growth rate and maximal biomass concentration achieved became saturated at a lower light intensity than did the growth rate and maximal biomass concentration for photoautotrophic cultures (Fig. 1). Mixotrophic cultures are also less sensitive to oversaturating light intensity (Fig. 2).

The higher dependency of photoautotrophic cultures on light is evident from the data presented in Fig. 2. Although mixotrophic cultures grew faster at any given light intensity (Fig. 2A), the response of photoautotrophic cultures to changes in light was more significant, either because of an increase to the light limited levels or inhibition at the higher light intensities.

Comparing the different parameters calculated from the P-I curves of the two cultures provides additional support for this observation. Although no significant difference in the photosynthetic efficiency (α) of the cultures is observed, all the other parameters associated with the response to light, such as the compensation point (E_c) or the light saturation value (E_k) are higher in the mixotrophic culture. This indicates that although the mixotrophic culture does not

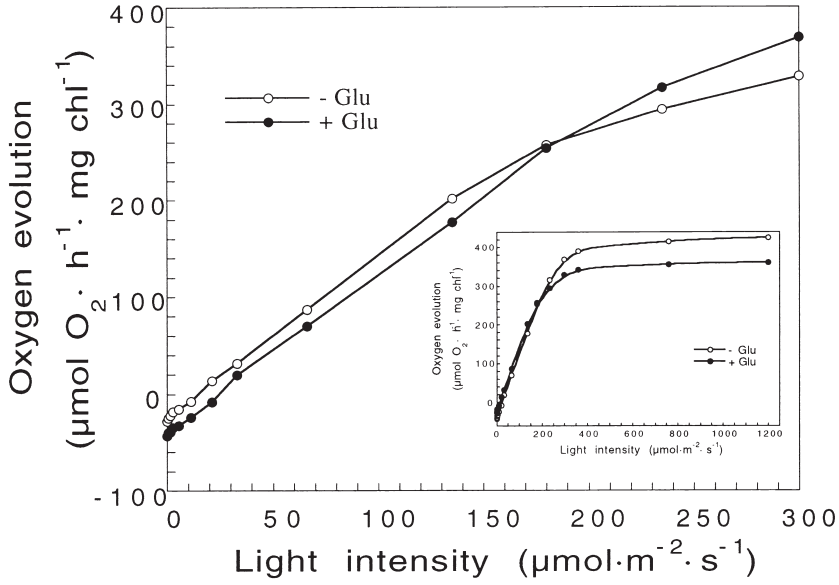

FIG. 3. Light response curve of mixotrophic and photoautotrophic cultures of *Spirulina platensis* in the low-light intensity range and the full-light intensity range (inset).

use light energy more efficiently at the lower intensity level, it is capable of using more light energy than the photoautotrophic culture.

It is generally accepted that photoinhibition results from an inability of the photosynthetic apparatus to use excess light energy absorbed by the photosynthetic antenna (for a review see Powels 1984, Kyle et al. 1987). One may hypothesize that the ability of using more light energy and having a higher saturation threshold of photosynthetic activity may result in lower susceptibility of the mixotrophic cells to light stress. This hypothesis is supported by our finding (Fig. 4), which shows when mixotrophically grown cells are exposed to HPFD, the maximal efficiency of PS II photochemistry and the overall light-dependent oxygen evolution are less impaired in those cells, compared with photoautotrophically grown cells. Both cultures are capable of recovering from the HPFD stress, yet the mixotrophic culture recovers faster than the photoautotrophic culture. The recovery from the photoinhibitory stress is not just a reaction process but requires an active metabolic process (Ohad et al. 1984). Thus, we suggest that the faster recovery rate observed in mixotrophic cultures is because of a higher metabolic activity. Dark respiration and the maximal photosynthetic activity, two parameters that may provide an indication for the metabolic activity of the cells, are significantly higher in the mixotrophic culture.

TABLE 1. Photosynthetic parameters of photoautotrophic and mixotrophic cultures of *Spirulina platensis*.[a]

Photosynthetic parameters	Growth conditions	
	Photoautotrophic	Mixotrophic
α	1.64 ± .042	1.58 ± .035
E_k	234 ± 6	291 ± 3
E_c	15 ± 1	25 ± 2
R_d	24 ± 4	41 ± 1
P_{max}	358 ± 6	420 ± 9

[a] α, initial slope of the P-I curve; R_d, dark respiration rate; E_c, compensation point; P_{max}, light-saturated photosynthetic rate; E_k, saturation irradiance. Values are the mean of three measurements ± SE. E_k and E_c are values of PFD given in μmol·m^{-2}·s^{-1}. R_d and P_{max} are given in units of rates of oxygen uptake or evolution in μmol O_2 h^{-1}·mg chl·h^{-1}.

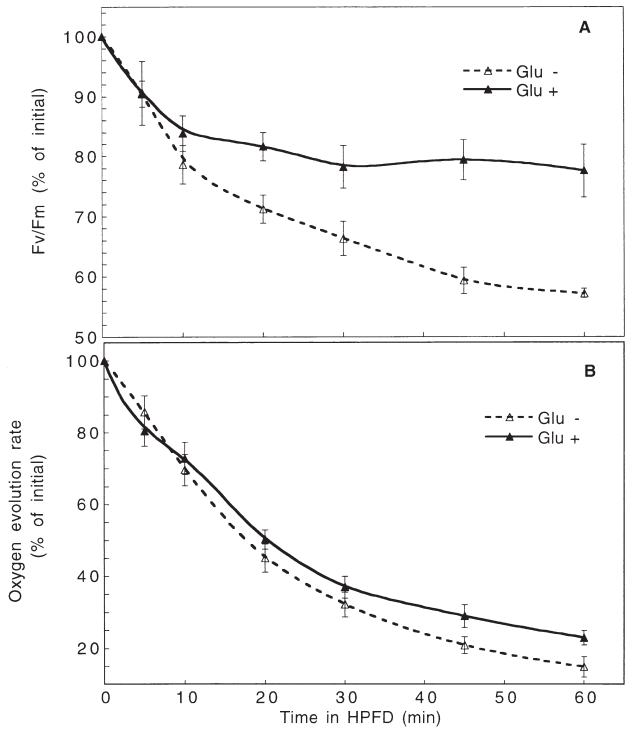

FIG. 4. The decline in the maximal photochemical efficiency of PS II (A) and oxygen evolution rate (B) of ▲ mixotrophic and △ photoautotrophic *Spirulina platensis* cultures exposed to HPFD of 3000 μmol·m^{-2}·s^{-1}.

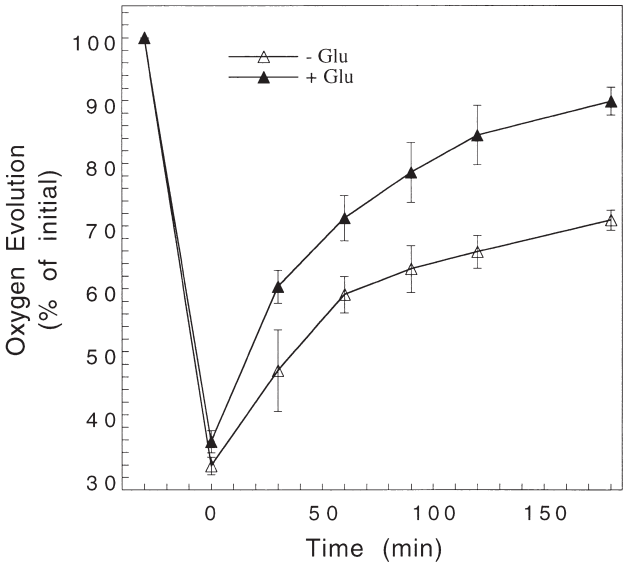

FIG. 5. Recovery of the oxygen evolution rate in ▲ mixotrophic and △ photoautotrophic *Spirulina platensis* cultures exposed to HPFD of 3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and then transferred to 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

In previous work, Marquez et al. (1993) demonstrated that *S. platensis* was able to grow heterotrophically in the dark or mixotrophically in the light in a nutrient medium enriched with glucose. The increase in cell concentration of the mixotrophic cultures corresponded to the sum of the autotrophic and heterotrophic cell concentrations, suggesting that autotrophic (photosynthesis) and heterotrophic (oxidative metabolism of glucose) growth functioned independently in mixotrophic cultures. The data presented in our work does not support this conclusion; rather, it suggests that the two metabolic processes do affect each other. Jones and Myers (1963) suggested that the respiratory activity and the photosynthetic activity of blue-green algae do have a common link. Schmetterer (1994) describes in detail the components of the electron transport chain of photosynthesis that are shared with the respiratory electron transfer chain, which include the cytochrome *b6f* complex. This observation further supports our conclusion that mixotrophic growth has a significant effect on photosynthetic activity and the response of the cyanobacterium *S. platensis* to light.

Bennet, J. & Bogorad, L. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell Biol.* 58:419–35.

Chen, F. & Zhang, Y. 1997. High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. *Enzyme Microbiol. Technol.* 20:221–4.

Chen, F., Zhang, Y. & Guo, S. 1996. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. *Biotechnol. Lett.* 18:603–8.

Ciferri, O. 1983. *Spirulina*, the edible microorganism. *Microbiol. Rev.* 47:551–578.

Endo, H., Sansawa, H. & Nakajima, K. 1977. Studies on *Chlorella regularis*, heterotrophic fast-growing strain. II. Mixotrophic growth in relation to light intensity and acetate concentration. *Plant Cell Physiol.* 18:199–205.

Guminski, S., Jurajda, K. & Tatkowska, E. 1985. Increase in the biomass of some green algae species in nitrate and ammonium media depending on auto-, mixo- or heterotrophic conditions. *Acta Soc. Bot. Pol.* 54:65–76.

Ignat′evskaya M-A, Thukral, A-K, Burdin, K-S & Nosov, V-N. 1996. Morphological and physiological characteristics of *Spirulina platensis* under conditions of photoautotrophic and mixotrophic growth in the light and dark. *Mikrobiologiya* 65:613–20.

Jones, L. W. & Myers, J. 1963. A common link between photosynthesis and respiration in the blue green algae. *Nature* 199:670–2.

Kobayashi, M., Kakizono, T., Nishio, N. & Nagai, S. 1992. Effects of light intensity, light quality, and illumination cycle on astaxanthin formation in a green alga, *Haematococcus pluvialis*. *J. Ferment. Bioeng.* 74:61–3.

Kyle, D. J., Osmond, C. B. & Arntzen, C. J. [Eds.] 1987. *Photoinhibition: Topics in Photosynthesis 9.* Elsevier Science Inc., Amsterdam, 315 pp.

Marquez, F. J, Nishio, N., Nagai, S. & Sasaki, K 1995. Enhancement of biomass and pigment production during growth of *Spirulina platensis* mixotrophic culture. *J. Chem. Tech. Biotech.* 62:159–64.

Marquez, F. J., Sasaki, K., Kakizono, T., Nishio, N. & Nagai, S. 1993. Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. *J. Ferment. Bioeng.* 76:408–10.

Marquez-Rocha, F. J. 1999. Reassessment of the bioenergetic yield of *Arthrospira platensis* using continuous culture. *World J. Microbiol. Biotechnol.* 15(2):235–8.

Misonou, T. & Pachlavuni, I. K. 1986. Photosynthetic pigments of Chlorella sp. K cultured under photoauto-, mixo and chemohetero-trophic growth conditions. *Jap. J. Phycol.* 34:163–70.

Ohad, I., Kyle, D. J. & Arntzen, C. J. 1984. Membrane protein damage and repair, removal and replacement of inactivated 32 kDa polypeptide in chloroplast membranes. *J. Cell Biol.* 99:481–5.

Powels, S. B. 1984. Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant. Physiol.* 35:15–44.

Rippka, R. 1972. Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. *Arch. Microbiol.* 87:93–8.

Schmetterer, G. 1994. Cyanobacterial respiration. *In* Bryant D. A. [Ed.] *The Molecular Biology of Cyanobacteria.* Kluwer Academic Publishers, Dordrecht, pp. 409–35.

Shamala, T. R., Drawert, F. & Leupold, G. 1982. Studies on *Scenedesmus acutus* growth. I. Effect of autotrophic and mixotrophic conditions on the growth of *Scenedesmus acutus*. *Biotech. Bioeng.* 24:1287–99.

Van-Kooten, O. & Snel, J. F. H. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Res.* 25:147–50.

Vonshak, A. (Ed.) 1997. *Spirulina platensis (Arthrospira): Physiology, Cell-Biology and Biotechnology.* Taylor & Francis, London, 233 pp.

Vonshak, A., Abeliovich, A., Boussiba, S. & Richmond, A. 1982. Production of *Spirulina* biomass: effects of environmental factors and population density. *Biomass* 2:175–84.