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The effect of light availability on the photosynthetic activity and productivity of outdoor cultures of *Arthrospira platensis* (*Spirulina*)

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Abstract The basic requirement for establishing economically viable large-scale production of algal biomass, be it for food, feed, high-value product, or energy, is the ability to produce the biomass at a low price. To achieve this goal, an efficient production protocol is needed that ensures that the potential productivity is obtained at any given time. When productivity is defined by the ability to utilize the available solar radiation that drives photosynthesis, the production protocol must be optimized to meet this requirement. In the current study, we demonstrate that by modifying the light available to *Arthrospira platensis* cells cultured outdoors by a variety of options like modifying the standing biomass concentration, changing the mixing rate, or shading can change the potential photosynthetic activity and apparent activity. By optimizing the light available to algae cells under outdoor conditions, productivity can be increased by approximately 50 %, from 15.6 g m^{-2} day^{-1} in a culture that suffers from overexposure to light to 22.4 g m^{-2} day^{-1} in a culture in which light downregulation is minimized. Therefore, by using a variety of methodologies to estimate photosynthetic activity, we demonstrate that overexposing the cells to light may result in downregulation of the photosynthetic activity leading to photoinhibition and lower biomass productivity.

Keywords Photosynthesis · *Arthrospira* · Outdoor · Photoinhibition

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Introduction

Algae cultures grown outdoors in raceway-shaped ponds are exposed to two different light/dark cycles. The first cycle, a relatively fast one, is induced by the mixing device causing a turbulent flow in the culture, the rate of which dictates the frequency of the light/dark cycle (Laws et al. 1988). Thus, the algae cells are shifted between being fully exposed to solar radiation when present at the upper culture surface to complete darkness when reaching the bottom of the culture, usually at a depth of 15–20 cm. The second cycle, a relatively slower one, results from changes in solar radiation during the day, from sunrise to sunset. These two light regimens impose unique physiological conditions in terms of the outdoor-grown algae cells acclimating to light from a highly light-limited situation up to a light-saturated stage that may result in photoinhibition or downregulation of the photosynthetic activity (Vonshak et al. 1982; Grobbelaar 1991, 1994; Vonshak and Guy 1992; Lu and Vonshak 1999).

The two main limiting factors for the growth of outdoor algae cultures that are nutrient-sufficient are generally accepted to be light and temperature. In relatively warm regions, where most algal biomass is produced, light has been assumed to be the main limiting factor for growth (Richmond and Vonshak 1978).

Many studies have attempted to increase the light available to algae cells grown outdoors. Richmond and Vonshak (1978) demonstrated an increase in the productivity of outdoor cultures of *Arthrospira platensis* by increasing the turbulent flow. This observation was confirmed by later studies (Laws et al. 1988; Fontes et al. 1991; Grobbelaar 1994; Sukenik et al. 2009). The explanation of this phenomenon is still being debated. Some of the explanations have attributed the result to improved mass transfer of nutrients, a flashing light effect (Kok 1956), and the removal of excess oxygen (Torzillo et al. 1998). However, at this stage, the increased turbulent flow in

outdoor algae cultures is generally accepted to improve the light/dark cycle, improving the photosynthetic efficiency of the cultures (Richmond 2004).

The mass production of *Spirulina* (*Arthrospira*) in outdoor open-raceway ponds has reached the commercial stage, with an estimated annual production of 8,000 MT. Further development of this industry is dependent on the ability to reduce production costs in order to allow the marketing of *Spirulina* as a feed additive rather than just a food additive in human nutrition. One way to achieve this goal is by increasing the productivity of outdoor cultures by overcoming some of the limiting environmental factors through better pond management and operation practices.

Since the somewhat simplified explanation of the role of light and temperature on outdoor algae cultures (Richmond and Vonshak 1978) that tried to attribute a single limiting factor to the growth and productivity of outdoor cultures (i.e., light in the summer and temperature in the winter), further studies have indicated that, in many cases, this is not necessarily the case. Outdoor algae cultures are exposed to diurnal fluctuations in light that may impose not only light limitations but also cause photoinhibition of the culture during a significant part of the day, preventing the culture from achieving maximum productivity (Vonshak and Guy 1992; Lu and Vonshak 1999). Although solar radiation is given for outdoor conditions, one can modify the light available to algae cells grown outdoors using different operational and technological parameters. We studied the response of the photosynthetic apparatus of *A. platensis* cells grown outdoors to induce changes in light availability. Using variable fluorescence techniques that were only recently introduced to the study of outdoor algae cultures (for review, see Masojidek et al. 2010) and provide a good measure of the maximal efficiency of photosystem II (PSII) photochemistry, as well as other online monitoring measurements, we offer insight into the role of light in dense, outdoor cultures of *A. platensis*.

Materials and methods

Arthrospira platensis strain M-2 was obtained from the Culture Collection of the Centro di Studio dei Microorganismi Autotrofi of Florence and grown outdoors in 2.5-m^2 ponds in Zarrouk's medium containing 200 mM sodium bicarbonate (Vonshak 1986). The culture depth was 12 cm. Mixing was performed by a paddle wheel at 7 or 17 rpm. The pH was maintained at 9.4 ±0.3 by the automatic addition of CO_2. Shading was provided by standard greenhouse shade nets that decrease the incident photon flux density (PFD) by 25 %.

Dry weight determination The culture (50 mL) was filtered through a GF/C filter (47 mm in diameter) and washed with an equal volume of acidified water (pH 4.0). Net productivity was calculated from the dry weight measurements of samples drawn out of the culture before and after the daily dilution.

Chlorophyll fluorescence measurement Variable chlorophyll fluorescence was used to estimate different activities in the photosynthetic apparatus. The most common parameters are F_o and F_m, which describe the minimal and maximal fluorescence yields of a dark-adapted sample with all PSII reaction centers fully open or closed, respectively. The ratio of the variable fluorescence ($F_v=F_m-F_o$) to maximal fluorescence (F_m) is interpreted as a measure of the maximal quantum efficiency of PSII photochemistry (for more nomenclature and definitions, see Van-Kooten and Snel 1990; Cosgrove and Borowitzka 2010). Algae samples (3 mL) were dark-adapted for 10 min in a liquid sample holder. Measurements were performed using a Plant Efficiency Analyzer fluorometer (PEA, Hansatech, UK).

Oxygen evolution The rate of oxygen evolution under light-limited conditions was measured in the lab using samples withdrawn from the outdoor-grown cultures, washed, and resuspended at a constant cell concentration (4 μg chl mL^{-1}) using a Clarke-type oxygen electrode in a double-jacket thermoregulated glass vessel at a constant temperature of 35 °C and under a PFD of 100 μmol photons m^{-2} s^{-1} provided by a slide projector lamp.

The O_2 evolution rate in the outdoor ponds was estimated by flashing the culture with a stream of N_2 to reduce the oxygen concentration to 100–110 % of saturation. The increase in O_2 concentration thereafter (5–10 min) was recorded and used to calculate the rate of O_2 evolution in the outdoor cultures as described previously (Ben-Yacov et al. 1985).

Electron transport rates Cells grown outdoors were harvested by centrifugation, washed twice with buffer (0.05 M Na_2HPO_4, 0.33 M mannitol pH 6.8), and resuspended in the assay buffer (0.33 M sorbitol, 50 mM HEPES, 5 mM $MgCl_2$, 10 mM NaCl, pH 7.8) to a final concentration of 25 μg chl mL^{-1}. The light-driven electron transfer rates of cells incubated at 35 °C under 800 μmol photons m^{-2} s^{-1} were estimated from the rate of O_2 evolution or O_2 uptake measured by a Clarke-type O_2 electrode. Artificial electron donors and acceptors were added immediately before illumination. The PSII activity, i.e., electron transfer from water to *p*-benzoquinone (pBQ), was determined by measuring the oxygen evolution rate in the presence of 0.9 mM pBQ. Photosystem I (PSI) activity was estimated by measuring the oxygen uptake in the presence of 0.10 mM reduced 2,6-dichlorophenol indophenol (DCPIP), 0.10 mM MV, 5 mM $NaNO_3$, 10 μM 3-(3,4-dichlorophynyl)1-dimethylurea (DCMU), 5 mM ascorbate, and 1 mM potassium cyanide. According to Robinson et al. (1982), intact *A. platensis* trichomes are permeable to the acceptors and donors used in our measurements. The chlorophyll concentration was determined

according to Bennet and Bogorad (1973). The algal biomass concentration in the ponds was estimated by measuring the optical density of the cultures at 560 nm. Light intensity was measured using a Li-Cor 185 photometer and quantum sensor.

Results

Effect of biomass concentration Light availability was modified by maintaining the cultures at different biomass concentrations (0.3 or 0.5 $OD_{560\ nm}$), which allowed light penetration to different culture depths based on different levels of self-shading in each culture. The maximal photochemical efficiency of PSII was followed by measuring the F_v/F_m ratio of the cultures. As shown in Fig. 1(a), the ratio decreased in the first few hours of the morning, starting soon after sunrise and reaching its lowest value at midday. A slow recovery occurred, and the F_v/F_m of the dense culture (0.5 $OD_{560\ nm}$) was reduced less than that of the less dense culture (0.3 OD_{560nm}) in which cells were exposed to higher light intensities.

Effect of shading Another way to modify the light available to each cell in an outdoor culture is by shading the ponds with a net that has a known light reduction value. An effect similar to that of the modification of biomass density was found when the light availability was modified by shading the 0.5 OD outdoor cultures with a net that removes 25 % of the total solar irradiance. The shaded culture exhibited a lower reduction in the F_v/F_m ratio compared to the unshaded culture at the same cell concentration (Fig. 1(b)). A recovery in the F_v/F_m ratio was observed after midday.

Effect of mixing The rate of turbulent flow does not affect the total light available to the cultures, but modifies the light/dark cycle that an algae cell undergoes. As demonstrated in Fig. 1(c), increasing the rate of turbulence from 7 to 17 rpm caused a similar effect as shading. At the higher turbulence, the reduction in the maximal photochemical efficiency of PSII observed at midday was significantly smaller than the reduction in the cultures with lower turbulent flow.

Effect of light availability on light-limited oxygen evolution rates Initial photoinhibitory damage in higher plants, as well as algae, can be detected by a reduction in the quantum efficiency of PSII. Thus, photoinhibition has more of an effect on the light-limited oxygen evolution rate than on the light-saturated activity (Henley 1993; Osmond 1994; Walker 1995). A similar response was demonstrated in photoinhibited *A. platensis* cells grown in the laboratory (Vonshak et al. 1989). Therefore, we followed diurnal changes in the light-limited photosynthetic activity (oxygen evolution rate) of *A. platensis* cultures grown outdoors under four different light conditions: at a constant biomass concentration defined by the maintenance of a constant turbidity of 0.3 or 0.5 OD with and without shading that reduced the total light energy at the culture surface by 25 %. Samples withdrawn during the day from the different outdoor ponds were washed and resuspended

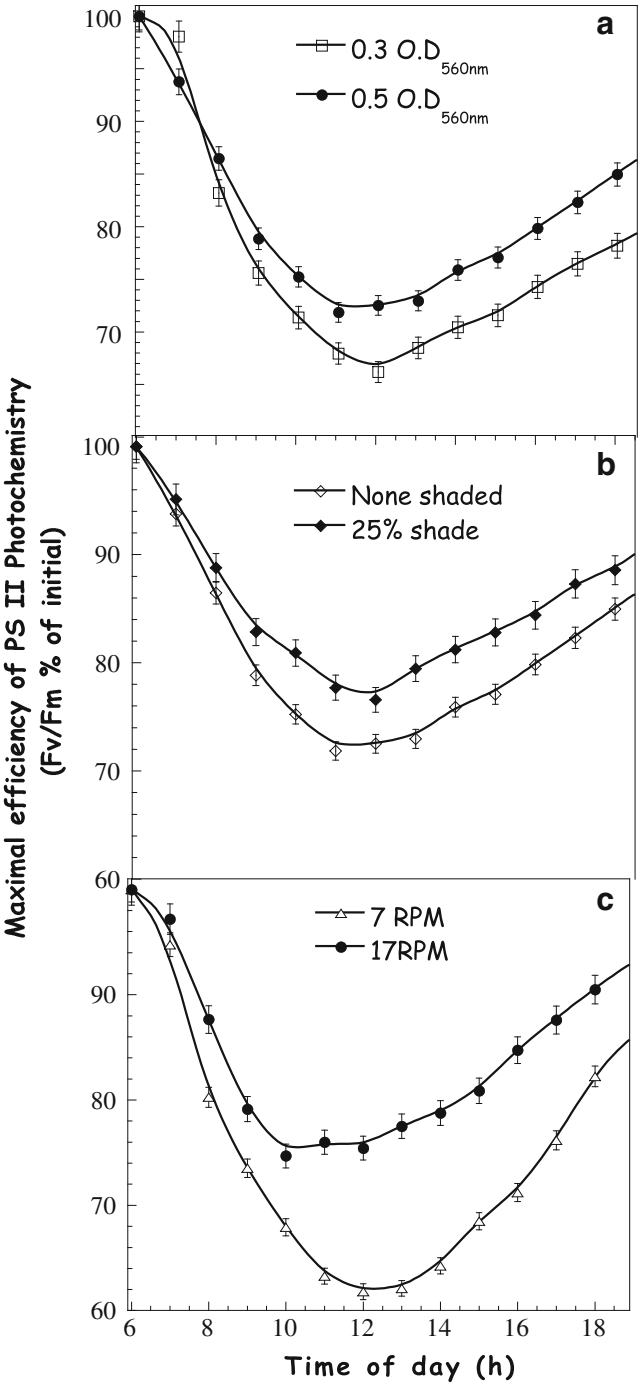

Fig. 1 Diurnal changes in the maximal photochemical efficiency of PSII in *A. platensis* cultures grown under different light conditions manipulated by (**a**) varying cell concentrations, (**b**) shading the culture with a net to reduce solar radiation by 25 %, and (**c**) varying the mixing level

in fresh medium to the same chlorophyll concentration and the light-limited oxygen evolution was determined. Figure 2 clearly demonstrates that the reduction in the light-limited oxygen evolution rate was greatest in the unshaded 0.3 OD culture at midday, when the cells were exposed to the highest irradiance. Decreasing the available light by increasing the culture density (0.5 OD), shading, or a combination of both reduced the decline in light-limited photosynthetic activity (Fig. 2). Importantly, the temperature of all cultures was the same throughout the day.

Effect of light availability on in situ photosynthetic activity Traditionally, O_2 concentration in algal ponds has been used to estimate the productivity or well-being of the cultures (Richmond and Vonshak 1978; Van der Heever and Grobbelaar 1997). However, the level of oxygen maintained in the culture is affected by a variety of additional parameters, which do not allow a direct correlation between oxygen concentration and photosynthetic activity, let alone productivity. The procedure developed by Ben-Yacov et al. (1985) allowed us to follow the in situ oxygen evolution rate of two *A. platensis* outdoor cultures that were maintained at the same cell concentration, one covered with a net providing 25 % shade and the other unshaded. As demonstrated in Fig. 3, in the early morning (6–10 a.m.), the O_2 evolution rate in the unshaded culture increased faster in response to the increase in solar radiation compared to the shaded culture, but later a decline was observed, demonstrating lower photosynthetic activity (O_2 evolution rate) compared to the shaded culture throughout the rest of the day. Thus, the photosynthetic activity in the shaded culture was significantly higher most of the day, even though it was exposed to lower solar radiation than the unshaded culture.

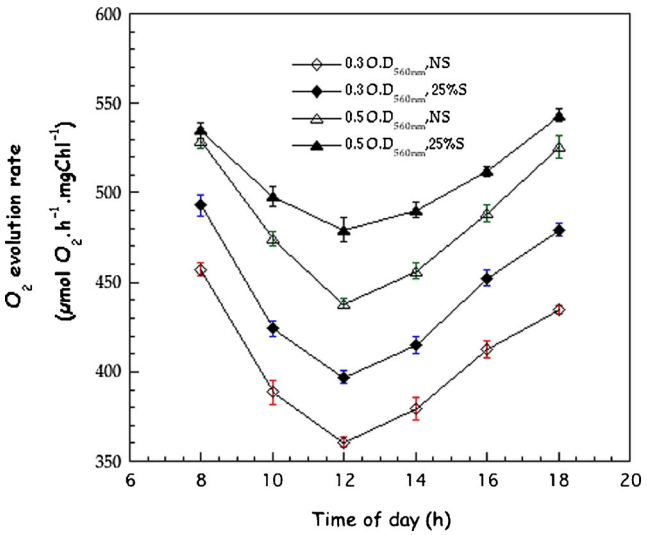

Fig. 2 Diurnal changes in the light-limited oxygen evolution rate of *A. platensis* cultures grown outdoors under different shading and light conditions manipulated by changing cell concentrations

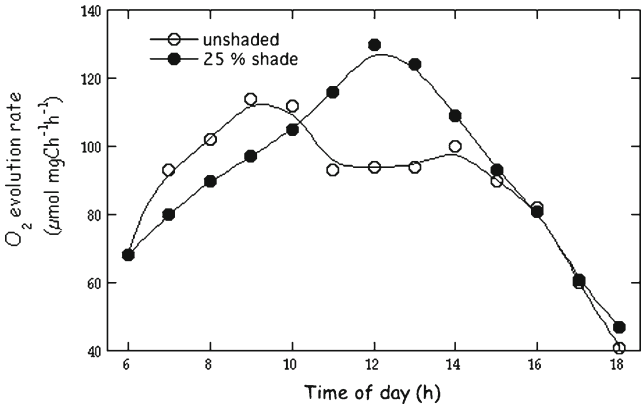

Fig. 3 Diurnal changes in the oxygen evolution rate in outdoor *A. platensis* cultures grown under two different light conditions modified by shading or not shading the cultures with a net that blocks 25 % of solar radiation

Effect of light availability on PSII and PSI activity The main site for downregulation or photoinhibition in higher plants and algae has been suggested to be PSII, whereas PSI activity is less affected (Demmig-Adams and Adams 1992; Murata et al. 2007; Öquist et al. 1995; Park et al. 1995). We followed changes in the partial electron transfer rates of PSII and PSI in outdoor cultures maintained at two different biomass concentrations, 0.3 and 0.5 OD_{560}, unshaded or shaded by a 25 % net throughout the day. PSII activity was significantly affected, displaying a significant decline at midday (Table 1), but no significant changes in PSI activity were observed during the day (Table 2).

Effect of light availability on productivity The daily productivity of the *A. platensis* cultures grown outdoors under the different light conditions was also recorded. Cultures were harvested daily by dilution to a constant biomass concentration, as indicated by the OD_{560}, and the total daily productivity was estimated. The highest productivity was achieved in the unshaded dense culture (0.5 OD_{560}) mixed at a rate of 17 rpm. Notably, the culture grown under conditions providing the highest light availability per cell (0.3 OD_{560}) had the lowest productivity.

Table 1 PSII activity of *A. platensis* cultures grown outdoors under different cell and shade conditions

Time of day	PSII activity 250 NS	PSII activity 250 25 % S	PSII activity 150 NS	PSII activity 150 25 % S
0600 hours	367.2	376.2	325.0	338.6
0800 hours	226.5	236.5	223.0	229.0
1200 hours	210.0	225.5	195.5	213.0
1300 hours	265.0	262.6	207.8	224.2
1700 hours	355.1	371.1	245.4	275.2

The cells were grown outdoors at different biomass concentrations (0.3 or 0.5 OD at 560 nm) with 25 % shading (S) or without shading (NS). Values are mean±SE of three independent measurements

Table 2 PSI activity of *A. platensis* cultures grown outdoors under different cell and shade conditions

Time of day	PSI activity 250 NS	PSI activity 250 25 % S	PSI activity 150 NS	PSI activity 150 25 % S
0600 hours	351.3	355.0	303.7	352.0
0800 hours	350.0	357.0	303.5	350.5
1200 hours	350.0	357.0	303.5	350.5
1300 hours	351.0	355.6	303.2	351.4
1700 hours	348.7	355.8	301.0	352.6

The cells were grown outdoors at different biomass concentrations (0.3 or 0.5 OD at 560 nm) with 25 % shading (S) or without shading (NS). Values are mean±SE of three independent measurements

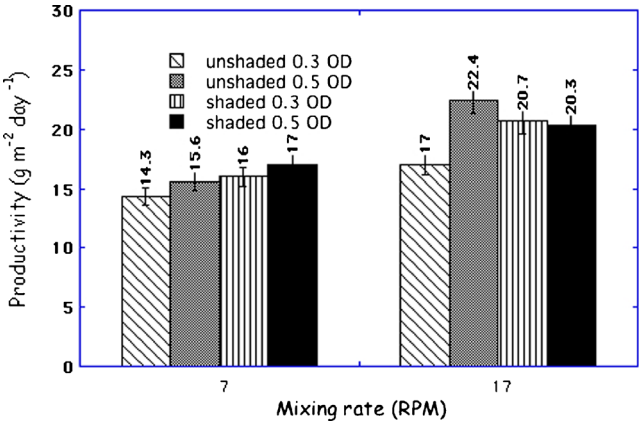

Fig. 4 The effect of mixing, shading, and cell concentration on the productivity of outdoor *A. platensis* cultures

Discussion

The effect of light availability on the maximal quantum efficiency of PSII photochemistry in *A. platensis* cells grown outdoors was studied by following the diurnal changes in the F_v/F_m ratio in cultures for which the light availability was modified by (1) changing the biomass concentration maintained in the ponds via daily dilution to a predefined constant concentration, (2) shading the culture with a shade net, or (3) modifying the turbulent flow of the culture. The objectives of the current study were to take advantage of the improved methodologies for measuring photosynthesis and the different procedures for evaluating the potential photosynthetic capacity in order to better understand the process governing growth and productivity in outdoor algae cultures. The effects of light and temperature on algae growth under controlled laboratory conditions have been intensively studied and reported. With the increased interest in mass algae cultivation in recent years, many of the productivity predications are based on these studies. The estimated productivity rates are greater than the achievable figures, mainly due to an underestimation of the particular stress conditions that prevail in outdoor cultures (Torzillo and Vonshak 2013). As mentioned previously, very few studies have evaluated the photosynthetic parameters of algae cultures grown outdoors, most using different methodologies of variable PSII fluorescence and some used a pass-through device to overcome the need to adapt to dark or constant light (Vonshak et al. 2001; Kromkamp et al. 2009; Sukenik et al. 2009). For the first time, this study reports the use of a previously developed methodology to directly estimate the oxygen evolution rate under a given temperature and light intensity.

Traditionally, light has been referred to as the main limiting factor in the mass culturing of microalgae (Burlew 1953; Grobbelaar et al. 1990, 1991; Richmond and Vonshak 1978; Richmond and Grobbelaar 1986). This assumption was based on the observation that outdoor algae cultures are kept in a dense suspension in which light penetrates only a small fraction of the culture. For practical reasons, none of the mixing devices used in such systems are able to induce a light/dark cycle that overlaps with the timescale of the flashing light effect demonstrated in the lab (Kok 1956). The most recent study that demonstrates again the effect of turbulence on productivity of dense algal cultures also indicates very high mixing rates that will be difficult to obtain in large-scale open ponds (Konk et al. 2013). The first to demonstrate that dense *Spirulina* (*A. platensis*) cultures grown outdoors undergo a photoinhibitory process were Vonshak and Guy (1992); they clearly demonstrated that, by shading the cultures and preventing them from being fully exposed to solar radiation, higher productivity can be maintained. At that time, no further evaluation procedures were employed to justify this conclusion.

Later, through the use of an in situ chlorophyll fluorescence technique, exposure to full sunlight was demonstrated to result in a midday decrease in the maximum quantum yield in dense *A. platensis* cultures grown under optimal conditions (Torzillo et al. 1996; Vonshak et al. 1996). This conclusion was supported by Lu and Vonshak (1999) using the polyphasic increase in chlorophyll fluorescence transients, which provide information on the primary photochemistry of PSII. The results suggest that the decrease in F_v/F_m observed in outdoor cultures was a result of an inactivation of the PSII reaction centers.

The use of variable fluorescence techniques to estimate the photosynthetic potential and activity of outdoor algae cultures was introduced relatively recently (Vonshak et al. 1994; Torzillo et al. 1998; Havlik et al. 2013) and is still not commonly used in many of the studies that try to identify the main limiting factors involved in algae cultivation outdoors. The potential of this methodology to provide useful information on the short-term and long-term changes in photosynthetic activity of outdoor algae cultures was further demonstrated by Kromkamp et al. (2009) and Sukenik et al. (2009) on outdoor *Nannochloropsis*.

In the current study, we combined the different approaches to reexamine the effect of light availability in an outdoor culture on the photosynthetic activity of algae cells and the overall performance of the culture as reflected in its productivity.

The data presented in Fig. 1 clearly indicate that a midday decline in the maximal photochemistry of PSII is the result of a high level of solar radiation. Reducing the exposure of the cells in the culture by increasing self-shading (Fig. 1(a)), increasing the mixing rate (Fig. 1(b)), or shading the culture (Fig. 1(c)) resulted in a reduced decline in the F_v/F_m at midday. For the first time, the oxygen evolution rates of cultures were measured and estimated during the day in an attempt to correlate them with light availability. The comparison between the apparent photosynthetic activity in outdoor shaded and unshaded cultures (Fig. 3) clearly demonstrates that the reduced potential of the photosynthetic activity (in F_v/F_m) was followed by a reduction in the apparent photosynthetic activity of the unshaded culture, inducing reduced productivity (Fig. 4).

The data presented in Tables 1 and 2 clearly demonstrate that PSII is much more affected than PSI as a function of light availability. The general agreement that photoinhibition mainly affects PSII provides further support for the hypothesis that outdoor algae cultures, if not well managed, may be photoinhibited.

The productivity of photoautotrophic algal biomass depends primarily on the light energy conversion efficiency (i.e., the absorption and utilization of light by the photosynthetic apparatus to assimilate CO_2 into dry matter). Therefore, dense algae cultures are grown predominantly under limited light; consequently, their photosynthetic activity is dependent on the initial slope (of the P–I curve) rather than the light-saturated portion of the P–I curve. Thus, a reduction in the F_v/F_m ratio due to excessive light absorption in the top layers of an outdoor algae culture results in decreased biomass yield. The data presented in Fig. 4 indicate the importance of using appropriate methodologies, such as mixing or devices that ensure a turbulent flow, in pond management in order to optimize the availability of light to outdoor cultures to increase biomass productivity.

Whatever the final product in the large-scale photoautotrophic mass culture of algae might be, the first requirement to achieve economic feasibility is the ability to optimize system productivity. First, one must identify the major limiting factors in order to ensure an optimal and efficient conversion of light energy to biomass. The aim of the work was to present the advantages of utilizing modern photosynthetic parameters in order to employ the correct pond/culture management methodologies to achieve this goal.

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