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Blooming of cyanobacteria in turbulent water with steep light gradients: The effect of intermittent light and dark periods on the oxygen evolution capacity of *Synechocystis* sp. PCC 6803

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Abstract

The influence of intermittent high-light dosage on *Synechocystis* sp. PCC 6803 with respect to oxygen evolution capacity, fluorescence yield and carotenoid pigment pattern was investigated, using high-light- and low-light-adapted cultures. The results showed that this cyanobacterium was able to survive high light stress for a full day if this stress was applied on and off with intermittently presented recovery periods in darkness. Enhanced respiratory activity in the high-light adapted cells was detected and this may be an important factor in preventing photodamage under high light stress. Cyanobacterial photosynthetic and respiratory electron transfer pathways are both present within the same membrane, and share common electron carriers. The role of respiratory activity in preventing overexcitation of photosystem 2 is discussed with regard to cyanobacterial ecology.

Keywords: Pigmentation; Light stress; Fluorescence; Recovery of photosynthesis

1. Introduction

Nowadays cyanobacteria frequently occur as massive blooms which give rise to environmental problems [1,2]. These mass developments are in the first order well correlated with an increase in the nutrient concentration in the water systems (the eutrophication process) [3,4]. However, this increase in the availability of nutrients can also favour other phytoplanktonic organisms [5,6]. Thus, eutrophication it-

self can only be the reason for the increase in the planktonic autotrophic biomass, but not for the selective promotion of cyanobacteria. A high affinity of the nutrient uptake systems of cyanobacteria, as shown by [7] and [8], may be the reason in systems with a temporary nutrient limitation, but many systems are so highly eutrophicated that during the whole year ample nutrient concentrations are always present. Moreover, [7] and [9] showed comparable high (P)-uptake capacities also for some Chlorophytes. Another possible explanation, the higher temperature optimum of some cyanobacteria, compared to the otherwise dominant green algae [10], can be used as an explanation for cyanobacteria

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being most dominant during the summer months, but recent work has shown that green algae with comparable high temperature optima also bloom in the same system [11].

A third important factor is the light regime. In aquatic ecology, cyanobacteria are mostly described as low-light-preferring organisms [12]. The reason for their dominance is explained by their high affinity for light, leading to relatively higher productivity under light-limited conditions [13] and by the generally lower maintenance energy needs in prokaryotes relative to eukaryotes [14]. Only a few laboratory experiments have been performed under conditions comparable to natural systems [15,16]. In a natural system light intensity (and quality, i.e. spectral composition) changes in various ways. The main change is of course imposed by the diurnal rhythm, modification of which occurs by actual weather conditions and season [17]. Additional vertical mixing processes induced by tides or wind, lead to a further influencing component with a shorter frequency. Even in shallow water systems these short-time vertical mixing processes should lead to a rhythmic exposure of the cyanobacteria to periods of very high light intensities which are ordinarily recognized to be lethal for these organisms [10,18,19]. Nevertheless, cyanobacteria appear to bloom in these systems as well. This raises questions on the influence of intermittent high light treatments on the photosynthesis of cyanobacteria.

2. Materials and methods

2.1. Cultivation

Two types of steady state continuous cultures of *Synechocystis* sp. PCC 6803 were used, both were grown in 2 l chemostats in BG-11 medium [20] at 20°C. One was grown at 25 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (low light, LL) the other one at 250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (high light, HL). Circular fluorescent tubes (Philips TLE 32W/33) were used for continuous illumination. The set-up of the culture system was as in [21]. Aeration at 60 l min^{-1} provided adequate mixing and CO_2 supply. The cultures were maintained at an OD_{750} of 0.16 to 0.18.

2.2. Pigment analysis

High performance liquid chromatography (HPLC) analysis was done as described in [22]. The specific extinction coefficients were taken from [22]. Samples were taken directly from the light exposure vessels and were immediately processed for pigment content estimation. This involved rapid centrifugation in a microfuge for precisely 1 min followed by mixing of the pellet with ice-cold acetone. Until the actual HPLC analysis was done, the samples were stored at -18°C .

2.3. OD_{750} and chl estimation

OD_{750} was measured on a Pharmacia Novaspec II photometer. Chlorophyll *a* (chl *a*) content was measured in acetone extracts [23].

2.4. Fluorescence measurements

Fluorescence parameters (F_m , maximal fluorescence, and F_s) were monitored with a pulse-amplitude modulated chlorophyll fluorescence measuring system (PAM, Walz, Germany) as described by [24]. During the measurements the fiber optic light-guide was directly placed against one side of the oxygen measuring chamber. With this method, oxygen production and fluorescence parameters could be estimated simultaneously. F_m was estimated every 120 s by firing saturating pulses of 500 ms duration (Schott KI-1500 light source, 12000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Data shown are the average of 3 separate experiments. Differences between comparable data points were below 8% of each.

2.5. Photoacoustic measurements

Photoacoustic measurements were done using a set-up as described in [25]. Far-red light for this measurement was given through a RG 695 cut-off filter. PSI Energy storage in far-red light only was estimated relative to a measurement in the presence of 1500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ white light to saturate photochemistry.

2.6. Photosynthetic oxygen evolution

P/I (oxygen evolution versus irradiance) curves were recorded according to standard procedures [26].

3. Results

The main parameters of the high-light (HL) or low-light (LL) steady-state continuous cultures used in this study are summarized in Table 1. The alpha (slope of the P/I curve at limiting irradiances) values (expressed on a per chl *a* basis) were nearly identical for both cultures, the standard deviations for $P_{\max}$ (maximum oxygen production rate) were overlapping and there was no statistically higher $P_{\max}$ for the HL-culture detectable. The phycocyanin content per cell was higher in the LL-culture than in the HL-culture. If calculated per unit chl *a*, the phycocyanin contents of both cultures were nearly identical. This effect has also been described by several other authors [27–29] under comparable cultivation conditions with other cyanobacterial strains. The carotenoid content per cell showed an increase of both xanthophylls and a slightly lower β -carotene content for the HL-cells. Expressed as a % of the initial content, the increase of zeaxanthin per cell was higher than that of myxoxanthophyll. However, when the β -carotene content is expressed relative to the chl *a* content per cell, the net result is an increase of the β -carotene per chl *a* ratio.

There was a correlation between fluorescence yield and oxygen evolution during the first hour, but

this correlation was not linear (Fig. 1). The fluorescence parameters changed very quickly, whereas oxygen evolution decreased more slowly after exposure to the highest irradiances.

The right hand parts of Fig. 1A and C show a gradual recovery to the original status of the fluorescence yield after termination of the light treatment and return of the samples to darkness. A relationship between the restoration kinetics, the intensity of the light stress and the original adaptation state (i.e. LL or HL grown) was evident. This dependency was more pronounced in the LL-culture than in the HL one. The original values were restored quite rapidly in most cases. This is in good agreement with the observation that α and $P_{\max}$ were restored in P/I curves measured after a restoration time of only 30 min (data not shown). Only very small changes in the $P_{\max}$ value were observed in the highest intensity treatments (max. 8% decrease in the 1250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ group). Any remaining observable differences in the fluorescence parameters after a restoration time of 30 min totally disappeared within 5 min of illumination with very low light intensities (data not shown). The 2400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ HL group and the 1250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ LL group remained impaired.

Fig. 2 shows changes in pigment content during periods of exposure to light stress. Some relative differences in the rate of disappearance of the pigments became apparent. During the first minutes, chl *a* and zeaxanthin are mainly affected, followed by a stabilization period. Changes in β -carotene and myxoxanthophyll, appeared later than those of chl *a*

Table 1
Photosynthetic oxygen evolution parameters and pigment content of LL- and HL-grown *Synechocystis* sp. PCC 6803

Light	Alpha	$P_{\max}$	Pigment contents	Chl <i>a</i>	Myxoxanthophyll	Zeaxanthin	β -Carotene
20 (LL)	0.23 (0.02)	44.2 (5.8)	81.2 (2.8)	12.1 (0.87)	4.06 (0.11)	1.86 (0.06)	2.23 (0.09)
250 (HL)	0.24 (0.03)	50.8 (6.2)	50.3 (3.4)	7.2 (0.68)	5.43 (0.09)	2.66 (0.08)	2.09 (0.09)

The averages of 4 independent determinations of each parameter are shown. Numbers in brackets are the standard deviations.

Units: alpha: $\text{mg O}_2 \text{l}^{-1} \text{h}^{-1} \text{mg chl } a^{-1} (\mu\text{mol photons m}^{-2} \text{s}^{-1})^{-1}$; $P_{\max}$: $\text{mg O}_2 \text{l}^{-1} \text{h}^{-1} \text{mg chl } a^{-1}$; pigment contents: $\text{fg} \cdot \text{cell}^{-1}$; light: adaptation light in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

and zeaxanthin. Zeaxanthin steadily disappeared during the full hour of the experiment.

There was a clear relation between the fluorescence recovery kinetics and the irradiance time at equal light intensity (Fig. 3). After short irradiance periods (5 min) there was a rapid recovery of variable fluorescence for HL, with complete recovery within 10 min of darkness. A biphasic kinetic of recovery was noticeable in the HL group. Longer exposure times resulted in a slower recovery of Fv/Fm, as well as a decrease in the role of the fast component. In LL cultures, the fast component of recovery was also less obvious, or was completely absent after irradiance treatments of more than 15 min.

These experiments showed that cyanobacteria were able to restore photosynthetic activity rapidly after a high light treatment, but that they were not

able to survive prolonged exposure to continuous light stress. A second set of experiments were performed to determine the amount of recovery present after increasing periods of light and dark. In these experiments the preadapted HL and LL cultures were exposed to light/dark periods at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ with different on/off frequencies. To allow direct comparisons, the cumulative exposure time (i.e. total dosage) over 6 h was equal for all treatments. Observed differences could only be caused by the frequency at which light and dark periods were intermittently presented. To prevent effects of differences in oxygen partial pressure between experiments, the oxygen electrode chambers were bubbled with air after each hour.

For both HL- and LL-cultured cells, the 15 min light/dark interval group showed no change in the rate of cumulative oxygen production during several

Fig. 1. Effects of exposure of *Synechocystis* sp. PCC 6803 to different light intensities on oxygen evolution and fluorescence yield. A and B: HL-adapted, C and D: LL-adapted, A and C: yield (Fv/Fm) B and D oxygen evolution (P_{max}) in % of the starting value (43.8 or 50.1 $\text{mg O}_2 \text{l}^{-1} \text{h}^{-1} \text{mg chl a}^{-1}$ for the LL- or HL-culture respectively) of the different light exposure treatments. Key to symbols: ■, 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; *, 430 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; ▲, 760 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; □, 1250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; +, 2400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Fig. 2. Changes in pigment content of *Synechocystis* sp. PCC 6803 during exposure to high light intensities. HL-grown cells treated with 1250 (A) and 2400 (B) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; LL-grown cells treated with 150 (C) and 760 (D) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Key to symbols: open bars, chlorophyll *a*; filled bars, myxoxanthophyll; hatched bars, zeaxanthin; striped bars, β -carotene.

hours. In the LL-adapted group the first decrease in productivity was detectable after 4 h, whereas in the HL-adapted cells the productivity started to diminish

slightly after 8 h. Exposure to longer time intervals resulted in a diminished cumulative O_2 production. The 30 and 45 min intervals gave similar results.

Fig. 3. Effects of duration of high light treatment on the recovery kinetics of F_v/F_m (yield) of *Synechocystis* sp. PCC 6803. A: HL-grown cells, treated with 2400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; B: LL-grown cells treated with 760 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for: 5 min ($\square$), 15 min ($\blacktriangle$), 30 min ($*$) and 60 min ($\blacksquare$), respectively.

After an initial period of net oxygen production, there was a decrease to zero after about 5 h in the LL-culture and after 9 h in the HL-culture. Net oxygen production reversed to net oxygen uptake for the last 2 or 3 h of the total 12 h of the experiment. The 60 min intervals gave a total accumulated rate of oxygen production higher than those found at the 30 and 45 min intervals, but lower than the 15 min value. In the case of the 60 min rhythm, no reversal to oxygen uptake was observed.

We questioned why the HL cells were able to perform better in all tests and investigated a physiological feature which is markedly different between chloroplasts and cyanobacteria. In the latter, a full respiratory chain is present which partially coincides with the photosynthetic electron transfer chain [30]. The impact of the respiratory chain on photosynthetic behaviour was studied by photoacoustic spectroscopy.

To estimate the gross effect of respiration on photosynthetic electron transport, KCN ($1 \mu\text{M}$) was added to inhibit the terminal cytochrome *c* oxidase. The differences in energy storage with and without cytochrome *c* oxidase activity amounted to 38% for HL-grown cells which was significantly ($\text{LSD} = 8.2$, $P \leq 0.05$) higher than the 29% for LL-grown cells (Fig. 4). This indicates that the terminal oxidase of the respiratory electron transport chain is more active in the HL cells than in the LL ones.

4. Discussion

Phototrophic organisms in wind-exposed, turbid water systems are exposed to intermittent high light and low light periods. The overall efficiency of conversion of light energy into biomass plays an important role in the competition between these organisms. Green algae, generally speaking, favour substantially higher light intensities than cyanobacteria [31]. The latter organisms have been considered as low light requiring under static conditions [12,32]. High solar irradiances encountered in summer should therefore favour blooming of green algae rather than cyanobacteria. Nevertheless, summer blooms of cyanobacteria are encountered. Explanations for these observations have included a better tolerance of higher temperatures [33] and a relative insensitivity to photoinhibition [34,35]. The work presented here addresses the question of how cyanobacteria tolerate periods of intermittent high light, as found in wind-exposed turbid water systems.

The data obtained by simultaneous measurement of fluorescence yield and oxygen evolution capacity showed a rapid decrease of photosynthesis upon exposure to high irradiance. Recovery upon return to darkness indicated that the changes in photosynthesis were due to down-regulation rather than photodestruction. The extent of recovery after exposure to high irradiance was dependent upon the length of the

Fig. 4. Accumulated oxygen production by *Synechocystis* sp. PCC 6803 under light/dark cycles presented at different frequencies. A: HL-grown cells; B: LL-grown cells. A light intensity of $1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was provided in the light periods throughout. Key to symbols: ■, 15 min dark/15 min light; +, 30 min dark/30 min light; ▲, 45 min dark/45 min light; □, 1 h dark/1 h light cycle. Y-axis: accumulated net oxygen production ($\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1} \text{ mg chl a}^{-1}$). Arrows indicate the isophoton points at 6 and 12 h.

irradiance period and upon the adaptation state of the algae.

Oxygen evolution, which responded more slowly to high light treatment, showed also a slower recovery kinetic in darkness (data not shown). Several mechanisms may explain the observed deviation between fluorescence yield and oxygen evolution. If photoinhibition (e.g. destruction of D1 protein) was involved, it should also be evidenced in the oxygen evolution capacity. Lack of apparent damage is in good agreement with the observations of [34,36,37] who demonstrated that *Synechocystis* sp. PCC 6803 has a very high turnover of the D1 protein. In agreement with other authors [35], we were not able to override this fast turnover mechanism and cause photoinhibition. Several other properties of cyanobacteria may also give protection against over-excitation of the photosynthetic electron transport chain. A comparatively large plastoquinone-pool and fast reoxidation of plastoquinone [38] would reduce the formation of potentially harmful long-lived excitation states in PSII. Fast reoxidation of the plastoquinone pool may proceed via autooxidation as in chlororespiration [39] or photorespiration [40] or via cytochrome *c* oxidase on the thylakoid membranes [41]. The latter pathway is unique to cyanobacteria. As the latter route potentially yields ATP [42], the interactions between photosynthetic electron transfer and respiratory pathways may thus be of additional advantage to cyanobacteria. Increased respiration has been discussed by Shyam et

al. [43] following photoinhibition in *Anacystis nidulans*.

In the present work we have extended this observation by showing that electron efflux via cytochrome *c* oxidase also occurs during illumination and is more important in HL cells than in LL cells (cf. Fig. 5).

In addition, protection against photoinhibition by carotenoids [44], especially by zeaxanthin was also demonstrated.

These results would indicate that cyanobacteria are able to survive periods of very high irradiance, particularly if they have been pre-adapted to moderate irradiance ($240 \mu\text{mol m}^{-2} \text{s}^{-1}$). This, however, is not the case in continuous irradiance conditions where high light exposure leads to cell death. We questioned why denser cultures of cyanobacteria can tolerate quite high light, whereas low density cultures are not able to survive. In other words, what role does mutual shading play and which periodicity of alternating high light and low light exposure allows *Synechocystis* to survive. Using the same net number of photons provided in a given period of time (6 h in this study) but presented as 15, 30, 45 or 60 min intense light exposures, each followed by an equal period of darkness, revealed clear differences in oxygen productivity. The shortest cycle (15 min) proved to be best. The survival period of the LL culture under fluctuating irradiance can be compared with natural conditions. Survival of 4 h would be sufficient to match sun exposure around noon. The

Fig. 5. 1/Energy storage versus far-red background light intensity spectra of *Synechocystis* sp. PCC 6803. A: HL-grown cells; B: LL-grown cells; ■, without addition; *, cells treated with 1 μM KCN.

HL-adapted culture sustained at least 8 h of intermittent high light exposure without noticeable losses of oxygen-evolving capacity.

In terms of competition, optimising the utilisation of light would provide an opportunity for the organism to obtain a competitive advantage. The ability of cyanobacteria to tolerate alternating light and dark periods in a 15 min on/off cycle via efficient and rapid PS II cross section adaptation, fast D1 repair, an extended plastoquinone pool size and an ATP yielding oxygen uptake pathway may provide this advantage. We hypothesise that green algae are better equipped to use higher light intensities in static conditions [45,46], but may still lose out in competition with cyanobacteria due to a faster loss of PS II activity through photoinhibition. Further investigations to exploit these observations for understanding formation of cyanobacterial blooms are in progress.

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