Biophysical Chemistry 182 (2013) 71–78

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Optimization of light use efficiency for biofuel production in algae

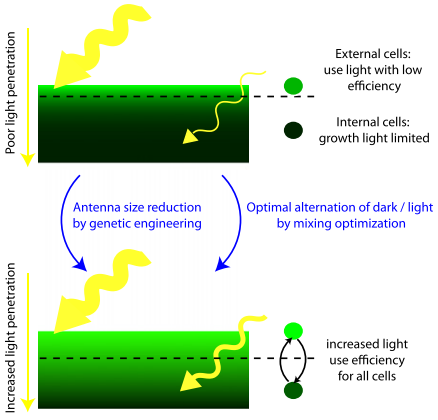
Diana Simionato, Stefania Basso, Giorgio M. Giacometti, Tomas Morosinotto *

Dipartimento di Biologia, Università di Padova, Via U. Bassi 58b, 35121 Padova, Italy

HIGHLIGHTS

- Algae have interesting potential for the production of biofuels.
- Light use efficiency is one of the major factors influencing algae productivity.
- Investigation of molecular bases influencing photochemical efficiency is seminal to optimize algae productivity.
- Productivity can be improved by genetic engineering and optimization of photobioreactor operational parameters.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form 20 June 2013
Accepted 21 June 2013
Available online 4 July 2013

Keywords:
Microalgae biofuels
Nannochloropsis
Photosynthesis
Photobioreactor
Pulsed light
Algae genetic engineering

ABSTRACT

A major challenge for next decades is development of competitive renewable energy sources, highly needed to compensate fossil fuels reserves and reduce greenhouse gas emissions. Among different possibilities, which are currently under investigation, there is the exploitation of unicellular algae for production of biofuels and biodiesel in particular. Some algae species have the ability of accumulating large amount of lipids within their cells which can be exploited as feedstock for the production of biodiesel. Strong research efforts are however still needed to fulfill this potential and optimize cultivation systems and biomass harvesting.
Light provides the energy supporting algae growth and available radiation must be exploited with the highest possible efficiency to optimize productivity and make microalgae large scale cultivation energetically and economically sustainable. Investigation of the molecular bases influencing light use efficiency is thus seminal for the success of this biotechnology. In this work factors influencing light use efficiency in algal biomass production are reviewed, focusing on how algae genetic engineering and control of light environment within photobioreactors can improve the productivity of large scale cultivation systems.

1. Introduction

The largest fraction of world energy demand is presently met by the combustion of coal, oil and natural gas. Such a massive exploitation of fossil fuels leads to the release of large amounts of carbon dioxide and other pollutants in the atmosphere with detrimental effects on the environment. Also, because of this massive consumption, global reserves will be depleted in the future. It is thus evident that there is a strong need of alternative, renewable and environmentally compatible sources of energy in order to sustain our present lifestyle [1]. Among different possibilities, photosynthetic organisms are receiving growing

* Corresponding author. Tel.: +39 0498277484; fax: +39 0498276300.
E-mail address: tomas.morosinotto@unipd.it (T. Morosinotto).

0301-4622/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bpc.2013.06.017

attention for their potential exploitation in the production of biofuels [2–6]. These bio-derived compounds in fact represent one of the most promising sources of liquid fuels, which are extensively used for transportation and in some cases, such as for jets, are not replaceable by electricity with the present technology.

In this direction, a major potential alternative to fossil fuels for transportation is biodiesel which can be produced from vegetal oil through a process of trans-esterification. Biodiesel production on a large scale, however, is at present strongly limited by the feedstock supply. Nowadays, most biodiesel is produced from oils extracted from crops like soy and palm, which have a limited productivity and would demand unrealistic areas of cultivation in order to replace a substantial fraction of fossil fuels [7,8]. A further critical point is that crop plants are normally employed as food or feed and their exploitation for biodiesel will generate an undesirable competition for cultivation areas and fresh water [9].

One interesting alternative to crops is the exploitation of other photosynthetic organisms such as microalgae, which are capable of accumulating large amounts of lipids which can be extracted, processed and refined into transportation fuels [4,6]. Algae also have additional interesting features such as the ability to efficiently use CO_2 [4] and, at least for some species, fast growth rate [10–14]. The production of biofuels can also be combined with the use of algal systems for wastewater treatment to reduce the carbon, nitrogen and phosphorus content in industrial, municipal and agriculture wastes [15,16]. Furthermore, microalgae-derived high added value molecules can be used in the cosmetic or food industry such as astaxanthin, β-carotene, omega-3-fatty acids, vitamin E and other pigments [16–19].

While it is thus clear in the scientific community that algae are highly promising for biofuel production and other applications, intensive research efforts are still needed to exploit their potential in large scale cultivation systems [5,6,20]. Many factors influence algae growth and productivity and deeper investigations are necessary to optimize operating parameters in large scale algae cultivation systems (photobioreactors, PBRs) and maximize their productivity (for a comprehensive review see [4,21]). One of the major factors affecting algae growth is light: as for all photosynthetic organisms, sunlight provides the energy supporting their metabolism and its efficient conversion into biomass has a major influence on productivity. The importance of this parameter is exemplified in Fig. 1, where the area needed to produce a ton of biomass per year is represented depending on the energy conversion efficiency. For values as low as 0.1%, the average value for most crop plants in field conditions [22], the requested area is very large, while this is drastically reduced if photosynthetic efficiency reaches 3%, a value experimentally obtained with algae in laboratory conditions [23]. Possible improvements could eventually further increase the biomass productivity, closing the gap with the maximal theoretical efficiency (77 ± 5 g dw m^{-2} d^{-1}, corresponding to ≈12% efficiency [24]).

It should be underlined that, while crop plants are routinely cultivated in large areas, algae are cultivated in photobioreactors or ponds which have high energetic and monetary cost for building and maintenance. Therefore any increase in the area occupied by the alga cultivation makes the process less sustainable from the energetic and economic point of view. Therefore, a high photosynthetic efficiency is indispensable for a viable algae large scale cultivation system, even more than for crop plants. For this reason a deeper understanding of the molecular bases of the light use efficiency for these organisms is seminal to optimize their cultivation on a large scale and will be the focus of the present work.

Although it is unlikely that a single species will have all the optimal characteristics for biodiesel production in all conditions, the species belonging to the genus *Nannochloropsis* are receiving increasing attention for this kind of applications. In fact, they present several positive features such as good growth rates and the ability to accumulate large amounts of lipids, up to 60% of total dry weight [14,25,26]. Recent

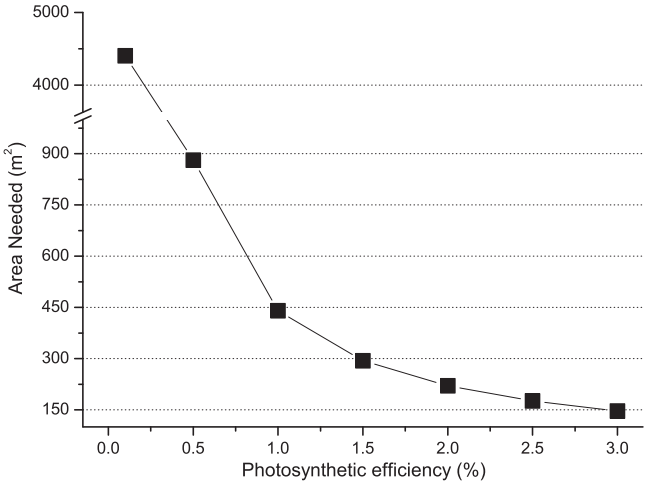


Fig. 1. Estimation of area needed for alga production. The area occupied by an alga cultivation system producing 1 ton of dry biomass per year is shown in dependence of the light use efficiency. Average radiation intensity was assumed to be 4541 MJ m^{-2} y^{-1} (data for Padova, Italy, according to Photovoltaic Geographical Information System, PVGIS Solar Irradiation Data, 2007, http://sunbird.jrc.it/pvgis/) and biomass energy content was assumed to be 20 kJ/g.

availability of genome sequences and tools for their molecular modification is also contributing to make this species a model for the study of biofuel production from algae [27,28], complementing the studies on other model organisms such as the green alga *Chlamydomonas reinhardtii* which is better characterized but less efficient as lipids producer. For this reason, *Nannochloropsis* and *Chlamydomonas* will be used here as the main reference species, although major conclusions are most likely valid for other species as well.

2. Influence of light intensity on photosynthetic efficiency

Algae grown in large scale cultivation systems, such as PBRs, are exposed to a complex light environment. First of all sunlight is not constant but its intensity continuously changes during the day and the seasons. Illumination intensity has an important influence on alga productivity, as shown in Fig. 2 for the case of *Nannochloropsis salina*: up to 150 μmol of photons m^{-2} s^{-1} an increase in illumination stimulates growth, showing that, in this range of intensities, available light is the limiting factor. Once this limit is surpassed, however, growth is not stimulated anymore by an increase in light intensity but, on the contrary, it has an inhibitory effect, causing reduction in duplication rate [29]. It is important to underline that, in the experiments reported here, *Nannochloropsis* cells were cultivated in a flat-bed PBR in order to expose all cells to the same irradiation, reducing as much as possible the cells' self-shading. Also, carbon dioxide and nutrients were provided in excess to highlight the influence of light regime on growth kinetics.

Similar experiments, performed at atmospheric CO_2 concentration, showed different results and light was limiting in a much smaller interval, only below 15 μmol of photons m^{-2} s^{-1} [30]. In these conditions, irradiation between 15 and 150 μmol of photons m^{-2} s^{-1} has little influence on duplication rates suggesting that, in this case, growth is limited by CO_2 supply (Fig. 2). The importance of this key substrate for algae growth is well established and in fact all large scale PBRs are normally designed to provide cells with additional CO_2 supply. Actually, the ability of algae to exploit high carbon dioxide supplies represents a major advantage of these organisms and opens the possibility of cultivating algae in connection with industrial processes which produce large amounts of CO_2. Such a combination, in fact, while providing a low cost CO_2 source for algae cultivation allows fixing part of it into biomass, thereby reducing emissions in the atmosphere.

For the above mentioned reasons, the influence of light regime on algae performances in a large scale PBR should be studied under

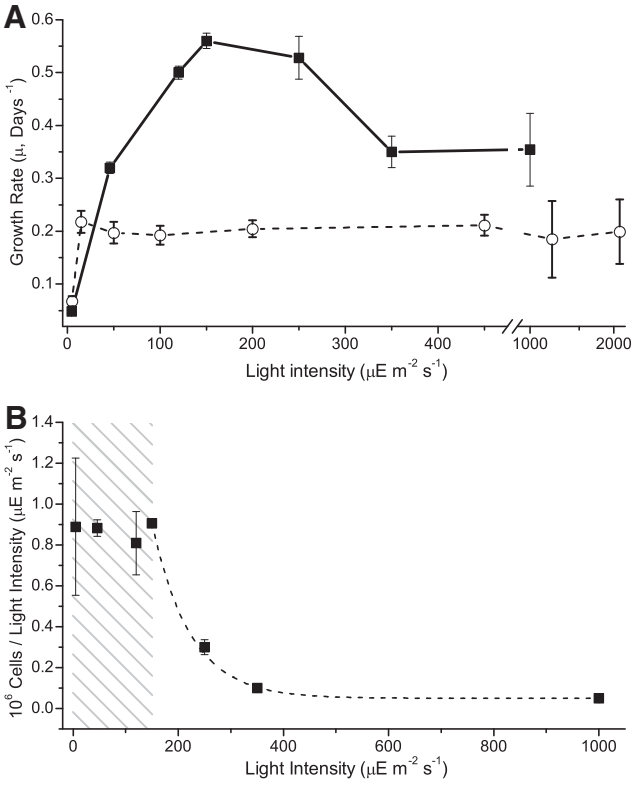


Fig. 2. Influence of light intensity on algae growth. A) *Nannochloropsis* growth, quantified as the specific growth rate calculated during the exponential phase under constant illumination is shown in dependence from light intensity (black squares). Light energy available increases linearly with the illumination intensity, as represented by the dotted line. CO_2 and nitrogen (as nitrate) were provided in excess to avoid growth limitation due to these nutrients and highlighting the influence of the light regime. B) cell concentration normalized to the light intensity, expressed as μE (μmol of photons) m^{-2} s^{-1}. Data reported are from [29].

conditions where CO_2 is in excess. When the effect of different light intensities is considered, it is important to underline that the amount of energy available grows linearly with the radiation intensity. Algae are highly efficient in harvesting light and even when exposed to a strong irradiation the culture still absorbs most of the available photons, meaning that the energy absorbed by the cells is also following a similar linear growth. Over 150 μE m^{-2} s^{-1}, however, an increase in the light available to the cells does not correspond to faster growth, implying that cells absorb energy in excess which they cannot use for biomass accumulation. This difference can be visualized by normalizing the growth to light intensity which allows an estimation of light use efficiency of the cultures. As shown in the first part of the curve in Fig. 2B, up to 150 μmol of photons m^{-2} s^{-1} this value is roughly constant, suggesting that in this range cells use light with a similar efficiency. When light intensity is over the maximal growth value, however, light use efficiency rapidly decreases. As an example, a comparison of data from 120 vs. 250 μmol of photons m^{-2} s^{-1} shows that while growth rate is similar, light use efficiency in the latter is already ≈50% less [29]. While data shown are referred to a specific set of experiments, a similar trend is observed for *Nannochloropsis* grown in cultivation systems with different geometries [31] or for other algae species [32–36], suggesting that the conclusions can be generalized.

Data reported above clearly show that light intensities over the saturation limit cause a drastic decrease in light use efficiency. Even if cells are still able to maintain a significant growth also under very intense illumination [29,30], in these conditions cells are highly inefficient in converting light into biomass. It is worth underlining that, while from the biological point of view there is no harm in using inefficiently an abundant resource, from the perspective of an alga large scale cultivation system any decrease in light use efficiency has a detrimental effect on system productivity, as shown in Fig. 1.

In order to devise strategies to keep algae productivity under a wider range of illumination, it is important to understand the molecular mechanisms responsible for the drop in light use efficiency under strong illumination. In photosynthetic eukaryotes most light is absorbed by pigments bound a family of proteins called antenna or light harvesting complex (LHC, [37]). Absorbed energy in the form of electronic excitation is transferred between nearby pigments and is eventually trapped by the special chlorophyll *a* contained in the reaction centers (RC) of the photosystems (PS). Here, electronic excitation drives a charge separation with one electron being transferred from the excited Chl *a* (Chl *a**) to a nearby acceptor molecule starting the electron transport chain which leads to ATP and NADPH synthesis.

Although light is indispensable to support algae metabolism, it may also become dangerous when in excess [32,33]. Light absorption and charge separation, in fact, take place in the presence of molecular oxygen. Toxic amount of ROS is formed in the thylakoid membrane when the absorption of light by chlorophylls exceeds the photosynthetic apparatus capacity of using excitation energy for electron transport, and photochemical reactions are saturated [38]. In particular, the very reactive singlet oxygen ($^1O_2$) can easily be created by light within the PSII complex in the presence of a photosensitizer such as chlorophyll, which is the main pigment of the photosynthetic apparatus [33,38]. Then, under conditions of intense illumination, excess energy leads to the production first of an increased amount of triplet excited state chlorophyll (^{3}Chl*) which in turn generates $^1O_2$ [39] that can easily oxidize and degrade pigments, proteins and lipids.

This ROS production under strong illumination has been suggested to impair PSII efficiency by inducing the degradation of some components of this protein–pigment complex (see a recent review by [40]). In cells exposed to strong illumination, the Photosystem II protein subunit D1 is continuously degraded and re-synthesized [32,41–43]. Although the molecular mechanism of reparation in vivo is not completely clarified, the damaged D1 subunit appears to be first removed from a photoinactivated PSII center through the progressive action of FtsH proteases [44,45] which bind the N-terminus of damaged D1 to drive its removal from the Photosystem II and its subsequent complete degradation [46,47]. After the removal of damaged D1 a new copy of the polypeptide is synthesized and re-inserted in PSII [44]. This repair mechanism is found conserved in all organisms performing oxygenic photosynthesis, from cyanobacteria [44] to plants [48], indicating that it plays a fundamental role in protection from irreversible photoinhibition. It has been estimated that D1 turnover in cells under illumination is around 30 minutes [32], and considering the abundance of PSII complexes in alga cells this implies that a relevant part of energy is invested in resynthesizing this protein. Although these mechanisms are clearly important to ensure cells survival, in the context of alga biomass production such a massive turnover clearly impair the efficiency of light conversion into biomass [43].

An additional strategy to cope with a strong illumination is the thermal dissipation of part of the energy absorbed by the antenna so as to balance the light capture to the photochemical reactions rate. Antenna complexes are responsible for most of the light harvesting, but they are also involved in this dissipation of excess excitation [49,50]. Energy absorbed by LHC, in fact, can be dissipated as heat before it reaches the RC reducing the amount of Chl excited states and consequently decreasing the probability of reactive oxygen species generation. This process is called Non Photochemical Quenching (NPQ) and it can dissipate as heat up to 80% of the total absorbed energy [33]. The NPQ is activated by strong light and it is present in all photosynthetic organisms starting from cyanobacteria to land plants, although the molecular mechanisms are variable in different organisms [50].

The fastest component of NPQ is activated a few seconds after exposition to strong illumination, when the accumulation of protons in the thylakoids lumen causes the protonation of PSBS in plants and LHCSR in algae [50–56]. When protonated, these proteins drive NPQ activation and the consequent decrease in excited state lifetime in pigment-binding subunits of the antenna system therefore reducing the possibility of generating high reactive singlet oxygen within PSII [57]. Carotenoids also play a major role in photoprotection and in all pigment–protein complexes of photosynthetic apparatus they are found close to chlorophylls, thus the sites for the potential production of triplet Chl and singlet oxygen. These carotenoid molecules act as quenchers for Chl excited states and also scavengers for reactive oxygen species eventually formed [58,59]. Carotenoids have different photo-protective abilities due to their specific chemical and structural properties, with zeaxanthin being particularly effective [58,60,61]. After exposition to strong light, zeaxanthin is synthesized from violaxanthin thanks to the activity of the enzyme violaxanthin de-epoxidase (VDE). This enzyme, at neutral pH, is in its monomeric inactive form in the lumen but, in high light, when the pH of this compartment decreases, it dimerizes, associates to the thylakoid membrane and converts violaxanthin into zeaxanthin [62,63]. This conversion increases cells' ability to quench ^{1}Chl*, ^{3}Chl* and $^1O_2$ [58,61].

A further mechanism contributing to photo-protection is 'state transition' which consists in the migration of LHCII, the PSII major antenna complex, from PSII (state 1) toward PSI (state 2) in order to equilibrate an imbalance of light excitation in the two photosystems. LHCII migration from PSII to PSI is activated upon phosphorylation when PSII is saturated and PQ pool is over-reduced. This is a reversible phenomenon and LHCII can migrate back to PSII when the excitation energy balance between the two photosystems is restored [64]. Although state transitions are present in both green algae and plants, its physiological role seems to be more important in the former [64]. In fact, in *Chlamydomonas*, the ability of activating state transitions was shown to contribute to a better carbon assimilation and algal growth [65]. Also, it was recently demonstrated in *Chlamydomonas* that cells' exposure to high light induce a persistent state 2 which contributes to reducing PSII functional antenna size and consequently protects this photosystem from over-excitation and in particular decreases hydrogen peroxide formation [66].

All mentioned mechanisms allow cells to safely dissipate energy in excess, thereby reducing oxidative damage, so as to survive under intense illumination [51]. In the perspective of algae large scale cultivation, however, energy dissipation as heat still causes a reduction of light use efficiency and has therefore a negative effect on culture productivity. Energy dissipated for protection, as well that used to repair the photosynthetic apparatus, strongly reduces biomass productivity and should be minimized in a large scale cultivation system [24,42].

3. Increase of light use efficiency by algae genetic engineering

An additional factor to be considered is that algae are cultivated in photobioreactors at high concentration and, because of the pigments present in the cells, the medium has a high optical density. As a consequence, light distribution in the systems is highly inhomogeneous [67–69] with the surface-exposed cells absorbing most of the available light, leaving only a residual part of the radiation for cells underneath (Fig. 3). For this reason, external layers are easily exposed to excess light and, as discussed above, to maintain their photosynthetic activity they need to dissipate energy and repair photoinhibited complexes with poor light use efficiency [24,70,71] (Fig. 3). At the same time, most of the cells in the culture are instead exposed to a weak illumination limiting their growth. If light is below the compensation point, cells might even have a negative productivity since respiration can be faster than photosynthesis. The relevance of this inhomogeneous light distribution on algae cultivation productivity is underlined by the observation that the overall efficiency of photobioreactors increases when the light path through the culture is shorter, reducing the inhomogeneity of light distribution [67,72–74]. Unfortunately, short light paths are difficult to be implemented in large-scale structures because of practical and economic reasons.

One possibility to reduce this limitation and increase productivity in algae large scale outdoor cultivation systems is to genetically engineer these organisms and make them more suitable to grow in the light environment found in a photobioreactor. In a natural environment, cells need to harvest light efficiently to compete with others and have no evolutionary advantage in leaving radiation energy to underneath layers. On the contrary, in a photobioreactor each cell should ideally only harvest the amount of light it can efficiently use for photochemistry. Unfortunately even if algae are a very diverse group of organisms it is unlikely that species isolated from the environment can have all the ideal characteristics for large scale cultivation and therefore their genetic improvement will likely be fundamental to optimize productivity [27,75]. Wild type (WT) algae thus need to be "domesticated", similarly to what happened for crops where multiple traits which would have a negative effect in a natural environment were artificially selected because, in cultivated crops, they provided a positive influence on productivity.

One of the possible strategies under intense study to improve light distribution in a photobioreactor is to generate strains with a decreased photosystems antenna size [36]. As mentioned above, antennas (or LHC) are pigment binding proteins which harvest light and transfer energy to the reaction centers. These proteins are particularly important in a light limiting environment because they increase the cells' ability to efficiently harvest available radiation. They also bind most of the pigments in algae cells and therefore are the main responsible for the optical density of the culture. Algae cells normally have a large number of antenna proteins associated to both Photosystems I and II and, for instance for *Chlamydomonas*, it has been estimated that there are respectively 200–240 and 190–210 chlorophylls per reaction center [76–78]. The presence of antenna proteins, however, is not necessary for electron transport reactions and their content can be reduced without affecting the photosystems' ability to perform photochemistry. It was estimated that the minimum number of Chl molecules is 37 for PSII and 95 for PSI, since below these limits the assembly of the photosystem core complexes is affected, impairing their photochemical activity [79]. However, these figures suggest that it is possible to strongly reduce the number of antenna proteins associated to each photosystem, which should have a positive effect on productivity in a photobioreactor by reducing the amount of pigments in each cell and improving light distribution, thereby increasing the energy available for all cells. Also cells with a small Chl antenna size would reach photosynthesis saturation at higher light intensity [71] reducing the amount of energy lost by cells at the surface of the photobioreactor.

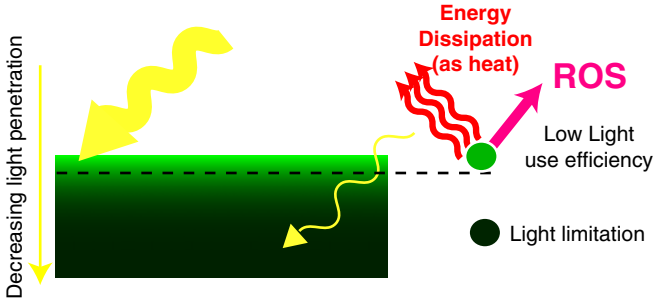


Fig. 3. Model of the light distribution in a photobioreactor. An algae photobioreactor has high optical density because of the high pigment concentration in the cells. A first layer of cells is thus exposed to full illumination and absorbs a major fraction of the light energy available. These cells likely have a saturated photosynthesis, leading to energy dissipation as heat and ROS production. As a consequence they not only absorb a large fraction of light available but they also use it with low efficiency. The rest of the biomass is left exposed to limiting light.

However, as mentioned above, antenna proteins are not only involved in light harvesting but also play a fundamental role in the protection from high light. In fact, thermal dissipation of energy absorbed in excess (NPQ described above) requires antenna proteins to be activated and mutants completely depleted of antenna proteins have been shown to be particularly sensitive to strong illumination. Thus, the desirable situation should not be a complete depletion of the antenna proteins but its selective reduction, which clearly requires a deeper knowledge of factor involved in photosystem biogenesis [80]. Following the domestication hypothesis, photosystems' antenna size must thus be re-optimized for photobioreactor conditions.

Different *C. reinhardtii* strains with reduced antenna size have already been isolated and characterized in the past few years using insertional mutagenesis and RNAi approaches [76,77,81–85]. *tla1* [77], *tla2* [76] and *tla3* [85] are DNA insertional transformants carrying mutations in genes involved in the modulation of expression of genes encoding for light harvesting complexes. As a consequence, these mutants have a truncated light harvesting chlorophyll antenna size and show a lower chlorophyll per cell content with respect to the corresponding WT strains. One interesting consequence of the truncated antenna size is the difference in the saturation of photosynthesis which occurs in *tla1* mutant at about 2500 μmol of photons m^{-2} s^{-1} instead of 1000 μmol of photons m^{-2} s^{-1} measured for WT [76,85].

Mutants with reduced antenna size were also obtained by RNA interference, exploiting the similarity between different LHC proteins to ensure the simultaneous down regulation of multiple genes. One line developed with this approach, called *StmLR3* [82], presents reduced levels of both LHCI and LHCII mRNAs and proteins. *StmLR3* shows a higher photosynthetic quantum yield and a reduced sensitivity to photo-inhibition which led to a faster cell growth under strong illumination. In fact, under illumination at 1000 μmol of photons m^{-2} s^{-1}, *Stm3LR3* cultures reached peak density already after 26.5 h when WT cultures only were at 54% of their maximal cell densities.

In conclusion, these works showed that the advantage of mutants with reduced antenna size is twofold. On one side, cells with truncated antenna, when exposed to strong irradiation, harvest light less efficiently, reducing the damage on the photosynthetic apparatus and the need to thermally dissipate absorbed energy. On the other side, light absorption by single cells in a mass culture is minimized allowing a better transmission in the culture thereby increasing overall photosynthesis and biomass accumulation. Both contributions yield into a higher productivity which for Tla1 at 1500 μmol of photons m^{-2} s^{-1} was estimated to be twice that of WT [77]. Although all present studies were performed with the model alga *C. reinhardtii*, it is expected that strains with a truncated antenna isolated for other species, more suitable for industrial applications, will yield similar advantages.

4. Light use efficiency increase through optimal alternation of dark/light cycles

Considering data reported in Fig. 2, a possible solution to grow algae with a good light use efficiency would be to cultivate them under light intensity below the saturation limit. However, illumination under a full sunlight in summer is over 2000 μmol of photons m^{-2} s^{-1}, suggesting that, for a large fraction of time, algae exposed to direct sunlight use energy with low efficiency, especially those located at the more external layers. However, this first layer of cells absorbs most of the available radiation and therefore this has a major negative effect for the overall productivity.

An additional factor of complexity to be also considered is that cells in a photobioreactor are actively mixed and move between dark and fully exposed regions of the photobioreactor [69]. The kinetics of mixing cycles vary greatly according to cultivation systems and change between a millisecond time-scale in closed tubular reactors or optical fiber-based photobioreactors to longer times by several order of magnitude in open ponds [69]. These dark/light cycles can strongly affect algae photosynthetic efficiency depending on the frequency and intensity of light flashes [86–91]. An example of their influence is shown in Fig. 4 where the growth of *N. salina* cells is reported when exposed to square-wave light/dark cycles to simulate mixing [29]. All experiments were performed providing the same total amount of photons, corresponding to 120 μmol of photons m^{-2} s^{-1} of continuous illumination, to evidence differences in light use efficiency due to frequency and intensity of light pulses [29]. In some conditions, the growth rate corresponds to that of cells exposed to constant moderate light (120 μmol of photons m^{-2} s^{-1}, Fig. 4A), suggesting that, in these conditions, cells were able to completely integrate the light absorption, exploiting intense light pulses as well as continuous illumination [92–94]. It is important to stress that this result implies that cells not only were able to avoid photo-oxidation damage under saturating flashes, but they were also able to use energy from pulses with the same efficiency as dim continuous light, even if they were 8 times over the saturation limit. Also, it is worth underlining that the intensity of the pulses was not affecting the results, at least in the range tested, since flashes of 350 and 1200 μmol of photons m^{-2} s^{-1} were exploited with the same efficiency.

The results with light flashes, however, were not always positive and in other cases growth was inhibited even if the integrated amount of photons provided was the same. The conditions showing the best productivity (1200 μmol of photons m^{-2} s^{-1} — 10 Hz and 350 μmol of photons m^{-2} s^{-1} — 30 Hz) had in common the same length of the light pulse (Fig. 4), which thus appears to have a large influence on biomass productivity among the parameters considered here. The optimal duration of light pulses was found to be around 10 ms [29] while longer pulses were not efficiently exploited. Similar results have been described for other species of microalgae [89,95] and with the model green alga *C. reinhardtii* it was shown that the specific growth rate under flashing light increased with the rise in flash frequency. Algae grown at 1000 μmol of photons m^{-2} s^{-1} — 100 Hz, again with 10 ms flash duration, presented similar growth rate and final biomass yield as the algae exposed to continuous 100 μmol of photons m^{-2} s^{-1}, confirming that the flash duration has a key influence on the light use efficiency [96,97]. These results suggest that a photobioreactor might well exploit with good efficiency even in highly intense light provided that mixing is optimized to that scope.

The timescale of the optimal duration of the light flashes, around 10 ms, is consistent with the suggested PSII turnover rate in whole cells [5,98], meaning that after charge separation by the photosystems, 10–15 ms are needed before the photosystem is ready to receive another photon [69]. In Fig. 4B a scheme providing a possible explanation for these observations is shown. One of the major rate limiting steps for photosynthesis is the Calvin–Benson cycle, which consumes ATP and NADPH produced by the light phase of photosynthesis for carbon fixation. Its activity has a fundamental influence also on the light phase because it re-generates the indispensable substrates ADP, Pi, and $NADP^+$. If light is too intense and Calvin–Benson cycle is not capable of fixing CO_2 at a sufficient rate, these substrates become limiting for the light phase of photosynthesis which, as a consequence, is not able to use all available energy for photochemistry, leading to the above discussed radiation damage and activation of heat dissipation mechanisms. In the case of experiments with pulsed light shown in Fig. 4A the total amount of energy provided is lower than the saturation point (120 vs. 150 μE m^{-2} s^{-1}) suggesting that the dark reactions should be able to use the energy with good efficiency and carbon fixation rate should not be limiting in these conditions.

Another limiting step for photosynthesis is electron transport between PSII and PSI via $Cytb_6f$ which requires the diffusion of plastoquinone in the membrane. PSII final acceptor is a plastoquinone molecule bound to Q_B site, which is reduced in around 1 ms. Once plastoquinol is formed, however, it must diffuse into the membrane and donate electrons to $Cytb_6f$ and another plastoquinone molecule

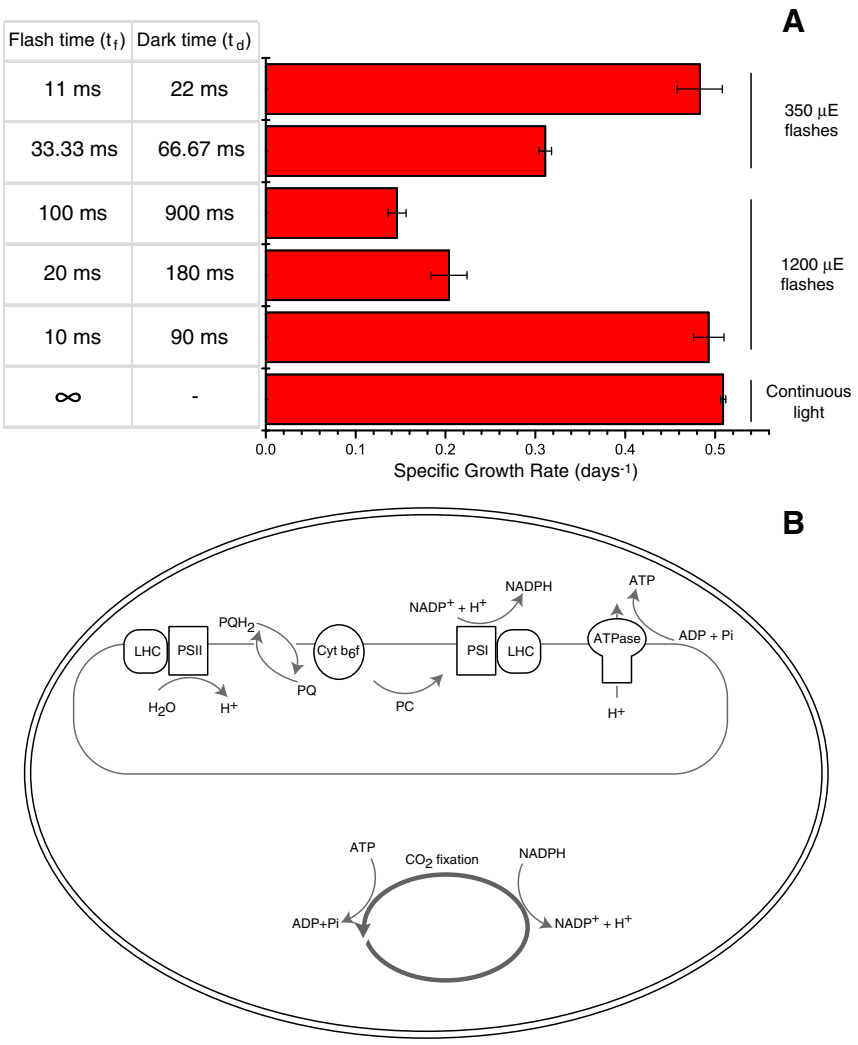


Fig. 4. Effect of pulsed light in algae growth. A) influence of alternation of light pulses and dark time on *Nannochloropsis* cell growth (data from [29]). All conditions provide a total amount of light corresponding to 120 µE m^{-2} s^{-1} of continuous light. CO_2 and nitrogen (as nitrate) were provided in excess to avoid growth limitation due to these nutrients and highlighting the influence of the light regime. B) Schematic representation of a chloroplast and main reactions of photosynthesis. Above the thylakoid membranes is shown together with the four protein super-complexes involved in the light phase of photosynthesis, PSII, $Cytb_6f$, PSI and ATPase. Some electron transport reactions are also indicated, water oxidation, diffusion of PQ between PSII and $Cytb_6f$, plastocyanin (PC) reduction and oxidation and $NADP^+$ reduction. Below is a schematic representation of Calvin–Benson cycle which consumes the products of light phase, ATP and $NADP^+$, regenerating the required substrates.

has to take its place in the Q_B site. This step is known to be a rate limiting step for the light phase of photosynthesis and in fact under intense illumination PSII is saturated at the level of PQ pool. Under light flashes plastoquinone is reduced but, if light is switched off fast enough, it allows the time for re-oxidation of electron transporters, thus preparing the reaction centers for the following pulse. If the light exposure is longer, instead, it increases the probability that a second photon reaches the reaction center when this is still in the oxidized state, thus leading to the generation of ROS and photo-damage. If the illumination is short enough, instead, not only the damage is reduced but also, since energy is "stored" as reduced PQ, the electron transport can proceed efficiently. In these conditions, the plastoquinone pool can act as a buffer which temporarily stores electrons allowing the efficient use of even very short light flashes.

These results suggest that very intense light can be harvested and exploited efficiently by cells growing in a photobioreactor, even if the total intensity is well beyond the saturation limit for that particular species. They also suggest that, provided that they are cultivated in optimal conditions, high photosynthetic efficiencies can be obtained also with algae growing in outdoor conditions exposed to very intense illuminations. However, for this to occur it is necessary that photobioreactor design is such that mixing is optimized and cells are exposed to short light pulses before moving back to the dark part of the photobioreactor. If this is possible, even very intense external light intensities could be harvested and used efficiently for photosynthesis.

Acknowledgments

This work was supported by FSE project from Regione Veneto, no. 2105/1/5/1739/2011 and ERC starting grant BIOLEAP no. 309485 to TM.

References

[1] J.L. Hong, Uncertainty propagation in life cycle assessment of biodiesel versus diesel: global warming and non-renewable energy, Bioresource Technology 113 (2012) 3–7.
[2] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, A. Darzins, Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances, The Plant Journal 54 (2008) 621–639.
[3] G.C. Dismukes, D. Carrieri, N. Bennette, G.M. Ananyev, M.C. Posewitz, Aquatic phototrophs: efficient alternatives to land-based crops for biofuels, Current Opinion in Biotechnology 19 (2008) 235–240.

[4] M. Hannon, J. Gimpel, M. Tran, B. Rasala, S. Mayfield, Biofuels from algae: challenges and potential, Biofuels 1 (2010) 763–784.
[5] F.X. Malcata, Microalgae and biofuels: a promising partnership? Trends in Biotechnology 29 (2011) 542–549.
[6] Y. Chisti, J.Y. Yan, Energy from algae: current status and future trends algal biofuels — a status report, Applied Energy 88 (2011) 3277–3279.
[7] G.T. Jeong, D.H. Park, C.H. Kang, W.T. Lee, C.S. Sunwoo, C.H. Yoon, B.C. Choi, H.S. Kim, S.W. Kim, U.T. Lee, Production of biodiesel fuel by transesterification of rapeseed oil, Applied Biochemistry and Biotechnology 113–116 (2004) 747–758.
[8] Y. Chisti, Biodiesel from microalgae, Biotechnology Advances 25 (2007) 294–306.
[9] A. Singh, P.S. Nigam, J.D. Murphy, Renewable fuels from algae: an answer to debatable land based fuels, Bioresource Technology 102 (2011) 10–16.
[10] M.K. Lam, K.T. Lee, Microalgae biofuels: a critical review of issues, problems and the way forward, Biotechnology Advances 30 (2012) 673–690.
[11] L. Gouveia, A.C. Oliveira, Microalgae as a raw material for biofuels production, Journal of Industrial Microbiology and Biotechnology 36 (2009) 269–274.
[12] G. Breuer, P.P. Lamers, D.E. Martens, R.B. Draaisma, R.H. Wijffels, The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains, Bioresource Technology 124 (2012) 217–226.
[13] C. Adams, V. Godfrey, B. Wahlen, L. Seefeldt, B. Bugbee, Understanding precision nitrogen stress to optimize the growth and lipid content tradeoff in oleaginous green microalgae, Bioresource Technology 131C (2012) 188–194.
[14] L. Rodolfi, Z.G. Chini, N. Bassi, G. Padovani, N. Biondi, G. Bonini, M.R. Tredici, Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, Biotechnology and Bioengineering 102 (2009) 100–112.
[15] L.L. Jiang, S.J. Luo, X.L. Fan, Z.M. Yang, R.B. Guo, Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO2, Applied Energy 88 (2011) 3336–3341.
[16] G. Sivakumar, J.F. Xu, R.W. Thompson, Y. Yang, P. Randol-Smith, P.J. Weathers, Integrated green algal technology for bioremediation and biofuel, Bioresource Technology 107 (2012) 1–9.
[17] L.M. Lubian, O. Montero, I. Moreno-Garrido, I.E. Huertas, C. Sobrino, M. Gonzalez-del Valle, G. Pares, *Nannochloropsis* (Eustigmatophyceae) as source of commercially valuable pigments, Journal of Applied Phycology 12 (2000) 249–255.
[18] J.A. Del Campo, M. Garcia-Gonzalez, M.G. Guerrero, Outdoor cultivation of microalgae for carotenoid production: current state and perspectives, Applied Microbiology and Biotechnology 74 (2007) 1163–1174.
[19] A.C. Guedes, H.M. Amaro, F.X. Malcata, Microalgae as sources of carotenoids, Marine Drugs 9 (2011) 625–644.
[20] H.M. Amaro, A.C. Guedes, F.X. Malcata, Advances and perspectives in using microalgae to produce biodiesel, Applied Energy 88 (2011) 3402–3410.
[21] V.H. Work, S. D'Adamo, R. Radakovits, R.E. Jinkerson, M.C. Posewitz, Improving photosynthesis and metabolic networks for the competitive production of phototroph-derived biofuels, Current Opinion in Biotechnology 23 (3) (Jun. 2012) 290–297.
[22] E.A. Heaton, R.B. Flavell, P.N. Mascia, S.R. Thomas, F.G. Dohleman, S.P. Long, Herbaceous energy crop development: recent progress and future prospects, Current Opinion in Biotechnology 19 (2008) 202–209.
[23] R.E. Blankenship, D.M. Tiede, J. Barber, G.W. Brudvig, G. Fleming, M. Ghirardi, M.R. Gunner, W. Junge, D.M. Kramer, A. Melis, T.A. Moore, C.C. Moser, D.G. Nocera, A.J. Nozik, D.R. Ort, W.W. Parson, R.C. Prince, R.T. Sayre, Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement, Science 332 (2011) 805–809.
[24] A. Melis, Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency, Plant Science 177 (2009) 272–280.
[25] S. Boussiba, A. Vonshak, Z. Cohen, Y. Avissar, A. Richmond, Lipid and biomass production by the halotolerant microalga *Nannochloropsis salina*, Biomass 12 (1987) 37–47.
[26] P.A. Hodgson, R.J. Henderson, J.R. Sargent, J.W. Leftley, Patterns of variation in the lipid class and fatty-acid composition of *Nannochloropsis oculata* (Eustigmatophyceae) during batch culture.1. The growth-cycle, Journal of Applied Phycology 3 (1991) 169–181.
[27] R. Radakovits, R.E. Jinkerson, S.I. Fuerstenberg, H. Tae, R.E. Settlage, J.L. Boore, M.C. Posewitz, Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*, Nature Communications 3 (2012) 686.
[28] O. Kilian, C.S. Benemann, K.K. Niyogi, B. Vick, High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. Proceedings of the National Academy of Sciences of the United States of America 108 (2011) 21265–21269.
[29] E. Sforza, D. Simionato, G.M. Giacometti, A. Bertucco, T. Morosinotto, Adjusted light and dark cycles can optimize photosynthetic efficiency in algae growing in photobioreactors, PLoS One 7 (2012) e38975.
[30] D. Simionato, E. Sforza, C.E. Corteggiani, A. Bertucco, G.M. Giacometti, T. Morosinotto, Acclimation of *Nannochloropsis gaditana* to different illumination regimes: effects on lipids accumulation, Bioresource Technology 102 (2011) 6026–6032.
[31] J. Van Wagenen, T.W. Miller, S. Hobbs, P. Hook, B. Crowe, M. Huesemann, Effects of light and temperature on fatty acid production in *Nannochloropsis salina*, Energies 5 (2012) 731–740.
[32] J. Barber, B. Andersson, Too much of a good thing: light can be bad for photosynthesis, Trends in Biochemical Sciences 17 (1992) 61–66.
[33] Z. Li, S. Wakao, B.B. Fischer, K.K. Niyogi, Sensing and responding to excess light, Annual Review of Plant Biology 60 (2009) 239–260.
[34] H. Qiang, A. Richmond, Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor, Journal of Applied Phycology 8 (1996) 139–145.
[35] K. Sakamoto, M. Baba, I. Suzuki, M.M. Watanabe, Y. Shiraiwa, Optimization of light for growth, photosynthesis, and hydrocarbon production by the colonial microalga *Botryococcus braunii* BOT-22, Bioresource Technology 110 (2012) 474–479.
[36] A. Melis, J. Neidhardt, J.R. Benemann, *Dunaliella salina* (Chlorophyta) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells, Journal of Applied Phycology 10 (1998) 515–525.
[37] A.G. Koziol, T. Borza, K. Ishida, P. Keeling, R.W. Lee, D.G. Durnford, Tracing the evolution of the light-harvesting antennae in chlorophyll a/b-containing organisms, Plant Physiology 143 (2007) 1802–1816.
[38] K. Asada, The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons, Annual Review of Plant Physiology and Plant Molecular Biology 50 (1999) 601–639.
[39] S. Takahashi, M.R. Badger, Photoprotection in plants: a new light on photosystem II damage, Trends in Plant Science 16 (2011) 53–60.
[40] I. Vass, Molecular mechanisms of photodamage in the Photosystem II complex, Biochimica et Biophysica Acta 1817 (2012) 209–217.
[41] P.J. Nixon, F. Michoux, J. Yu, M. Boehm, J. Komenda, Recent advances in understanding the assembly and repair of photosystem II, Annals of Botany 106 (2010) 1–16.
[42] J.A. Raven, The cost of photoinhibition, Physiologia Plantarum 142 (2011) 87–104.
[43] I. Szabo, E. Bergantino, G.M. Giacometti, Light and oxygenic photosynthesis: energy dissipation as a protection mechanism against photo-oxidation, EMBO Reports 6 (2005) 629–634.
[44] P. Silva, E. Thompson, S. Bailey, O. Kruse, C.W. Mullineaux, C. Robinson, N.H. Mann, P.J. Nixon, FtsH is involved in the early stages of repair of photosystem II in *Synechocystis* sp. PCC 6803, The Plant Cell 15 (2003) 2152–2164.
[45] D.A. Campbell, E. Tyystjarvi, Parameterization of photosystem II photoinactivation and repair, Biochimica et Biophysica Acta 1817 (2012) 258–265.
[46] J. Komenda, R. Sobotka, P.J. Nixon, Assembling and maintaining the Photosystem II complex in chloroplasts and cyanobacteria, Current Opinion in Plant Biology 15 (2012) 245–251.
[47] M. Lindahl, C. Spetea, T. Hundal, A.B. Oppenheim, Z. Adam, B. Andersson, The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein, The Plant Cell 12 (2000) 419–431.
[48] S. Bailey, E. Thompson, P.J. Nixon, P. Horton, C.W. Mullineaux, C. Robinson, N.H. Mann, A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle in vivo, Journal of Biological Chemistry 277 (2002) 2006–2011.
[49] M. Ballottari, J. Girardon, L. Dall'Osto, R. Bassi, Evolution and functional properties of photosystem II light harvesting complexes in eukaryotes, Biochimica et Biophysica Acta 1817 (2012) 143–157.
[50] K.K. Niyogi, T.B. Truong, Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis, Current Opinion in Plant Biology 16 (3) (Jun. 2013) 307–314.
[51] G. Peers, T.B. Truong, E. Ostendorf, A. Busch, D. Elrad, A.R. Grossman, M. Hippler, K.K. Niyogi, An ancient light-harvesting protein is critical for the regulation of algal photosynthesis, Nature 462 (2009) 518–521.
[52] A. Alboresi, C. Gerotto, G.M. Giacometti, R. Bassi, T. Morosinotto, *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization, Proceedings of the National Academy of Sciences of the United States of America 107 (2010) 11128–11133.
[53] G. Bonente, M. Ballottari, T.B. Truong, T. Morosinotto, T.K. Ahn, G.R. Fleming, K.K. Niyogi, R. Bassi, Analysis of LhcSR3, a protein essential for feedback de-excitation in the green alga *Chlamydomonas reinhardtii*, PLoS Biology 9 (2011) e1000577.
[54] S.L. Mou, X.W. Zhang, N.H. Ye, M.T. Dong, C.W. Liang, Q. Liang, J.L. Miao, D. Xu, Z. Zheng, Cloning and expression analysis of two different LhcSR genes involved in stress adaptation in an Antarctic microalga, *Chlamydomonas* sp. ICE-L, Extremophiles 16 (2012) 193–203.
[55] S. Cao, X. Zhang, D. Xu, X. Fan, S. Mou, Y. Wang, N. Ye, W. Wang, A transthylakoid proton gradient and inhibitors induce a non-photochemical fluorescence quenching in unicellular algae *Nannochloropsis* sp. FEBS Letters 587 (2013) 1310–1315.
[56] C. Gerotto, T. Morosinotto, Evolution of photoprotection mechanisms upon land colonization: evidences of PSBS dependent NPQ in late streptophyta algae, Physiologia Plantarum (May 10 2013), http://dx.doi.org/10.1111/ppl.12070, (in press).
[57] G. Bonente, B.D. Howes, S. Caffarri, G. Smulevich, R. Bassi, Interactions between the photosystem II subunit PsbS and xanthophylls studied in vivo and in vitro, Journal of Biological Chemistry 283 (2008) 8434–8445.
[58] I. Baroli, A.D. Do, T. Yamane, K.K. Niyogi, Zeaxanthin accumulation in the absence of a functional xanthophyll cycle protects *Chlamydomonas reinhardtii* from photooxidative stress, The Plant Cell 15 (2003) 992–1008.
[59] C. Triantaphylides, M. Havaux, Singlet oxygen in plants: production, detoxification and signaling, Trends in Plant Science 14 (2009) 219–228.
[60] M. Eskling, P.-O. Arvidsson, H.-E. Akerlund, The xanthophyll cycle, its regulation and components, Physiologia Plantarum 100 (1997) 806–816.
[61] J.P. Connelly, M.G. Müller, R. Bassi, R. Croce, A.R. Holzwarth, Femtosecond transient absorption study of carotenoid to chlorophyll energy transfer in the light-harvesting complex II of photosystem II, Biochemistry 36 (2) (Jan. 14 1997) 281–287.
[62] P. Arnoux, T. Morosinotto, G. Saga, R. Bassi, D. Pignol, A structural basis for the pH-dependent xanthophyll cycle in *Arabidopsis thaliana*, The Plant Cell 21 (2009) 2036–2044.

[63] C. Fufezan, D. Simionato, T. Morosinotto, Identification of key residues for pH dependent activation of violaxanthin de-epoxidase from *Arabidopsis thaliana*, PLoS One 7 (2012) e35669.
[64] F.A. Wollman, State transitions reveal the dynamics and flexibility of the photosynthetic apparatus, EMBO Journal 20 (2001) 3623–3630.
[65] P. Cardol, J. Alric, J. Girard-Bascou, F. Franck, F.A. Wollman, G. Finazzi, Impaired respiration discloses the physiological significance of state transitions in *Chlamydomonas*, Proceedings of the National Academy of Sciences of the United States of America 106 (2009) 15979–15984.
[66] G. Allorent, R. Tokutsu, T. Roach, G. Peers, P. Cardol, J. Girard-Bascou, D. Seigneurin-Berny, D. Petroutsos, M. Kuntz, C. Breyton, F. Franck, F.A. Wollman, K.K. Niyogi, A. Krieger-Liszkay, J. Minagawa, G. Finazzi, A dual strategy to cope with high light in *Chlamydomonas reinhardtii*, The Plant Cell 25 (2013) 545–557.
[67] N. Zou, A. Richmond, Light-path length and population density in photoacclimation of *Nannochloropsis* sp. (Eustigmatophyceae), Journal of Applied Phycology 12 (2000) 349–354.
[68] A.M. Kunjapur, R.B. Eldridge, Photobioreactor design for commercial biofuel production from microalgae, Industrial and Engineering Chemistry Research 49 (2010) 3516–3526.
[69] A.P. Carvalho, S.O. Silva, J.M. Baptista, F.X. Malcata, Light requirements in microalgal photobioreactors: an overview of biophotonic aspects, Applied Microbiology and Biotechnology 89 (2011) 1275–1288.
[70] A. Melis, Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage ? Trends in Plant Science 4 (1999) 130–135.
[71] Y. Nakajima, R. Ueda, Improvement of microalgal photosynthetic productivity by reducing the content of light harvesting pigment, Journal of Applied Phycology 11 (1999) 195–201.
[72] A. Richmond, Z. Cheng-Wu, Y. Zarmi, Efficient use of strong light for high photosynthetic productivity: interrelationships between the optical path, the optimal population density and cell-growth inhibition, Biomolecular Engineering 20 (2003) 229–236.
[73] C. Posten, G. Schaub, Microalgae and terrestrial biomass as source for fuels—a process view, Journal of Biotechnology 142 (2009) 64–69.
[74] C.Y. Chen, K.L. Yeh, R. Aisyah, D.J. Lee, J.S. Chang, Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review, Bioresource Technology 102 (2011) 71–81.
[75] R.E. Jinkerson, R. Radakovits, M.C. Posewitz, Genomic insights from the oleaginous model alga *Nannochloropsis gaditana*, Bioengineered 4 (2013) 37–43.
[76] H. Kirst, J.G. Garcia-Cerdan, A. Zurbriggen, A. Melis, Assembly of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* requires expression of the TLA2-CpFTSY gene, Plant Physiology 158 (2012) 930–945.
[77] J.E. Polle, S.D. Kanakagiri, A. Melis, tla1, a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size, Planta 217 (2003) 49–59.
[78] A. Melis, Spectroscopic methods in photosynthesis: photosystem stoichiometry and chlorophyll antenna size, Philos. Trans. R. Soc. Lond. B 323 (1989) 397–409.
[79] R.E. Glick, A. Melis, Minimum photosynthetic unit size in system I and system II of barley chloroplasts, Biochimica et Biophysica Acta 934 (1988) 151–155.
[80] C. Formighieri, F. Franck, R. Bassi, Regulation of the pigment optical density of an algal cell: filling the gap between photosynthetic productivity in the laboratory and in mass culture, Journal of Biotechnology 162 (2012) 115–123.
[81] J.H. Mussgnug, L. Wobbe, I. Elles, C. Claus, M. Hamilton, A. Fink, U. Kahmann, A. Kapazoglou, C.W. Mullineaux, M. Hippler, J. Nickelsen, P.J. Nixon, O. Kruse, NAB1 is an RNA binding protein involved in the light-regulated differential expression of the light-harvesting antenna of *Chlamydomonas reinhardtii*, The Plant Cell 17 (2005) 3409–3421.
[82] J.H. Mussgnug, S. Thomas-Hall, J. Rupprecht, A. Foo, V. Klassen, A. McDowall, P.M. Schenk, O. Kruse, B. Hankamer, Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion, Plant Biotechnology Journal 5 (2007) 802–814.
[83] S.D. Tetali, M. Mitra, A. Melis, Development of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* is regulated by the novel Tla1 gene, Planta 225 (2007) 813–829.
[84] G. Bonente, C. Formighieri, M. Mantelli, C. Catalanotti, G. Giuliano, T. Morosinotto, R. Bassi, Mutagenesis and phenotypic selection as a strategy toward domestication of *Chlamydomonas reinhardtii* strains for improved performance in photobioreactors, Photosynthesis Research 108 (2–3) (Sep. 2011) 107–120.
[85] H. Kirst, J.G. Garcia-Cerdan, A. Zurbriggen, T. Ruehle, A. Melis, Truncated photosystem chlorophyll antenna size in the green microalga *Chlamydomonas reinhardtii* upon deletion of the TLA3-CpSRP43 gene, Plant Physiology 160 (2012) 2251–2260.
[86] S. Xue, Z. Su, W. Cong, Growth of *Spirulina platensis* enhanced under intermittent illumination, Journal of Biotechnology 151 (2011) 271–277.
[87] J.M. Gordon, J.E. Polle, Ultrahigh bioproductivity from algae, Applied Microbiology and Biotechnology 76 (2007) 969–975.
[88] J.N. Phillips, J. Myers, Growth rate of *Chlorella* in flashing light, Plant Physiology 29 (1954) 152–161.
[89] H.C. Matthijs, H. Balke, U.M. van Hes, B.M. Kroon, L.R. Mur, R.A. Binot, Application of light-emitting diodes in bioreactors: flashing light effects and energy economy in algal culture (*Chlorella pyrenoidosa*), Biotechnology and Bioengineering 50 (1996) 98–107.
[90] Z.H. Kim, S.H. Kim, H.S. Lee, C.G. Lee, Enhanced production of astaxanthin by flashing light using *Haematococcus pluvialis*, Enzyme and Microbial Technology 39 (2006) 414–419.
[91] K.H. Park, C.-G. Lee, Effectiveness of flashing light for increasing photosynthetic efficiency of microalgal cultures over a critical cell density, Biotechnology and Bioprocess Engineering 6 (2001) 189–193.
[92] F.C. Rubio, F.G. Camacho, J.M. Sevilla, Y. Chisti, E.M. Grima, A mechanistic model of photosynthesis in microalgae, Biotechnology and Bioengineering 81 (2003) 459–473.
[93] K.L. Terry, Photosynthesis in modulated light: quantitative dependence of photosynthetic enhancement on flashing rate, Biotechnology and Bioengineering 28 (1986) 988–995.
[94] K.H. Park, C.-G. Lee, Optimization of algal photobioreactors using flashing lights, Biotechnology and Bioprocess Engineering 5 (2000) 186–190.
[95] L. Nedbal, V. Tichy, F.H. Xiong, J.U. Grobbelaar, Microscopic green algae and cyanobacteria in high-frequency intermittent light, Journal of Applied Phycology 8 (1996) 325–333.
[96] C. Vejrazka, M. Janssen, M. Streefland, R.H. Wijffels, Photosynthetic efficiency of *Chlamydomonas reinhardtii* in attenuated, flashing light, Biotechnology and Bioengineering 109 (2012) 2567–2574.
[97] C. Vejrazka, M. Janssen, M. Streefland, R.H. Wijffels, Photosynthetic efficiency of *Chlamydomonas reinhardtii* in flashing light, Biotechnology and Bioengineering 108 (12) (Dec. 2011) 2905–2913.
[98] Z. Dubinsky, P.G. Falkowski, K. Wyman, Light harvesting and utilization by phytoplankton, Plant & Cell Physiology 27 (1986) 1335–1349.