J Appl Phycol (2015) 27:1063–1077
DOI 10.1007/s10811-014-0427-y

Antenna size reduction as a strategy to increase biomass productivity: a great potential not yet realized

Tim de Mooij · Marcel Janssen · Oscar Cerezo-Chinarro · Jan H. Mussgnug · Olaf Kruse · Matteo Ballottari · Roberto Bassi · Sandrine Bujaldon · Francis-André Wollman · René H. Wijffels

Received: 26 June 2014 / Revised and accepted: 6 October 2014 / Published online: 19 October 2014

Abstract A major limitation in achieving high photosynthetic efficiency in microalgae mass cultures is the fact that the intensity of direct sunlight greatly exceeds the photosynthetic capacity of the cells. Due to the high pigment content of algal cells, the light absorption rate surpasses the much slower conversion rate to biochemical energy. The excess of light energy is predominantly dissipated as heat, decreasing the light use efficiency of the culture. Algae with a truncated antenna system could substantially increase biomass productivity of mass cultures because oversaturation of the photosystems and concomitant dissipation of light energy are minimized. In this study, we measured the areal biomass productivity of wild-type strain cultures and four promising antenna size mutant cultures of *Chlamydomonas reinhardtii*. This was performed under simulated mass culture conditions. The strains were cultivated in turbidostat controlled lab-scale panel photobioreactors at an incident light intensity of 1500 μmol photons m^{-2} s^{-1}. The mutant cultures did not exhibit the expected higher productivity. The greatest mutant culture productivity values were approximate to those of the wild-type productivity of 1.9 g m^{-2} h^{-1}. The high sensitivity to abrupt light shifts indicated that the mutant cultures experienced reduced fitness and higher susceptibility to photodamage. This can possibly be explained by impaired photoprotection mechanisms induced by the antenna complex alterations, or by unintended side effects of the genetic modifications. Still, if these effects could be eliminated, the principle of antenna size reduction is a promising strategy to increase productivity. Selection criteria for the future creation of antenna size mutants should, therefore, include tolerance to high light conditions.

Keywords *Chlamydomonas reinhardtii* · Areal biomass productivity · Photosynthetic efficiency · Antenna size mutants · Biomass yield on light energy

Introduction

Cultivation of microalgae occurs in open race-way ponds and photobioreactors exploiting sunlight as an energy source. To achieve an economically viable process, high sunlight energy to biomass conversion efficiencies are crucial as they will decrease the production costs of microalgal biomass (Norsker et al. 2011). In relationship to biology, the absorption of light energy is the first step in the photosynthetic process and determines the degree of saturation of the photosynthetic apparatus. At approximately 10 % of full sunlight, photosystems already become saturated. If more light is absorbed, the excess light energy is mainly dissipated as heat or re-emitted as fluorescence. Oversaturation of photosynthesis leads to efficiency losses and photodamage of the photosystems (Deblois et al. 2013; Melis 1999; Vejrazka et al. 2013) and

Electronic supplementary material The online version of this article (doi:10.1007/s10811-014-0427-y) contains supplementary material, which is available to authorized users.

T. de Mooij (✉) · M. Janssen · O. Cerezo-Chinarro · R. H. Wijffels
Bioprocess Engineering, AlgaePARC, Wageningen University, PO box 16, 6700 AA Wageningen, The Netherlands
e-mail: tim.demooij@wur.nl

J. H. Mussgnug · O. Kruse
Algae Biotechnology & Bioenergy Group, Department of Biology, Center for Biotechnology, Bielefeld University, 33615 Bielefeld, Germany

M. Ballottari · R. Bassi
Department of Biotechnology, University of Verona, 15, Strada Le Grazie, 37134 Verona, Italy

S. Bujaldon · F.-A. Wollman
Institut de Biologie Physico-Chimique, Unité Mixte de Recherche 7141, Center National de la Recherche Scientifique/Université Pierre et Marie Curie, 75005 Paris, France

preventing oversaturation thus would increase the biomass yield on light energy.

The primary reason that microalgae cannot be cultivated at a high photosynthetic efficiency under outdoor conditions is the non-homogeneous distribution of sunlight in photobioreactors. Cells near the reactor surface become oversaturated due to being exposed to the intense sunlight, resulting in dissipation of excess light energy. By improving the light energy distribution within the reactor, the effects of oversaturation are alleviated. To date, the concept of light dilution is a frequently employed approach to optimize photobioreactor performance by increasing its surface area per unit of reactor volume (Cuaresma et al. 2011; Dye 2010; Tredici and Zittelli 1998; Zemke et al. 2013) whereby the light use efficiency can be increased. However, increasing the surface area to volume ratio of photobioreactors is complex and is often accompanied by increased material costs. Alternatively, with respect to the biological characteristics of microalgae, there continue to be opportunities for the improvement of light use efficiency.

An alternative approach to light dilution is to genetically engineer the microalgae cells to reduce the size of their antenna complexes (Formighieri et al. 2012; Kwon et al. 2013; Melis 2009; Mussgnug et al. 2007; Nakajima and Ueda 1998; Oey et al. 2013; Ort et al. 2011; Perrine et al. 2012). Antennae are light-harvesting systems that are evident in all known photosynthetic organisms (Grossman et al. 1995). They comprise protein-pigment complexes located in, or on, photosynthetic membranes. With genetic modification, the pigment content of the antennae can be reduced. These so-called antenna size mutants are more transparent and are light saturated at higher light intensities than wild-type cells. Antenna size mutants absorb less light per cell, however, so conversion of solar energy to chemical energy is expected to occur at a higher efficiency as direct sunlight is no longer oversaturating.

From an evolutionary perspective, individual cells benefit from an extensive antenna system because the natural habitat of many microalgae species can be relatively dark. In addition, wasting excess light energy can be advantageous as it is a way to outcompete other photosynthetic organisms, by shading them. Conversely, under mass culture conditions, it is not the individual cell productivity but, instead, the productivity of the culture in its entirety that should be optimized. Therefore, in a controlled mass culture, metabolic costs associated with competition strategies should be minimized. By truncating the light-harvesting antennae, cells will not experience oversaturation by light and, consequently, no light energy will be wasted. However, as this provides a competitive disadvantage, maintaining a monoculture of these mutants is of crucial significance.

Studies have demonstrated that, at light intensities that oversaturate wild-type cells, dilute cultures of antenna size mutant have greater photosynthetic activity (i.e., higher oxygen production rate) per unit of chlorophyll (Kirst et al. 2012; Nakajima and Ueda 2000; Perrine et al. 2012). These findings, however, cannot be translated to the potential of antenna size mutants under mass culture conditions. Since a mass culture possesses a very steep light gradient due to the high biomass density, the light regime in the photobioreactor is completely different. In addition, if measurements are expressed based on chlorophyll without providing the chlorophyll content itself, extrapolation to areal biomass productivity values ($g\ m^{-2}\ h^{-1}$) is complicated. As there is no linear relationship between light absorption and chlorophyll content and because chlorophyll is not the only light-harvesting pigment in green algae, expressing the biomass productivity per unit of light absorbance would have been more appropriate and a more valuable benchmark of the efficiency at which light energy is utilized. Another argument in opposition to oxygen evolution experiments is that they originate from short-term measurements (a time scale of minutes), indicating that it cannot be evaluated whether there is a progressive negative effect of the excessive light intensity on the cells in the long term.

In this study, we present an approach to quantitatively assess the long-term areal biomass productivity of microalgae cultures under simulated mass culture conditions. We employed this approach in order to evaluate the most promising antenna size mutants that are currently available. These mutants were selected based on the characterization and preliminary experiments performed by the developers of the strains, encouraging scale-up to mass culture conditions (Bonente et al. 2011; Kirst et al. 2012; Mussgnug et al. 2007). The performance of the mutants was compared to wild-type reference strains by measurement of the areal biomass productivity in continuous bench-scale culture systems. Under nutrient replete conditions, the areal biomass productivity of an algal culture is determined by the amount of light intensity that it is exposed to and the efficiency at which light energy is converted into biomass. The applied incident light intensity was comparable to direct sunlight, and the biomass concentration was maintained constant utilizing turbidostat control. The applied biomass concentration was sufficient to absorb all incoming light. By combining algal productivity with light absorption rates, the biomass yield on light energy was calculated. This biomass yield on light energy of antenna size mutants and the wild-type strain was employed to evaluate the potential of genetically engineered microalgae with a truncated antenna system in mass cultures.

Materials and methods

Organisms and medium

Chlamydomonas reinhardtii CC-1690, CC-124, and TLA2 were obtained from the *Chlamydomonas* Resource Center (University of Minnesota). More information regarding

TLA2 and the other mutant strains of interest (BF4, Stm3LR3, AS2.2) is shown in Table 1 and the indicated publications. All of these antenna size mutants possess partially assembled light-harvesting complexes (LHC) and as a result lack pigments located in the peripheral antenna complexes. The algae were cultivated in a Sueoka high salt (HS) medium with the following composition (in g L^{-1}): urea, 1.48; KH_2PO_4, 0.706; K_2HPO_4, 1.465; $MgSO_4 \cdot 7H_2O$, 0.560; $CaCl_2 \cdot 2H_2O$, 0.114; and 20 mL L^{-1} of a 100 times concentrated Hutner's trace elements solution (Hutner et al. 1950). The medium was filter-sterilized (pore size 0.2 μm) and was designed to sustain biomass concentrations up to 9 g L^{-1}. The cultures were pre-cultivated in 250-mL shake flasks containing 100 mL of medium at pH 6.7, at a light intensity of 200–300 μmol photons m^{-2} s^{-1}, and at 25 °C. Cultures were mixed with magnetic stirring bars.

Photobioreactor setup

The strains were continuously cultivated in flat-panel airlift photobioreactors (Algaemist, Technical Development Studio, Wageningen University, the Netherlands) with a working volume of 0.4 L, an optical depth of 14 mm, and an illuminated area of 0.028 m^2 (see Fig. 1 for a schematic overview). Light was provided by LED lamps (BXRA W1200, Bridgelux, USA) with a warm-white light spectrum (Fig. 2). Reactor temperature was adjusted to 25 °C, and the pH was maintained at 6.7 (±0.1) utilizing an on-demand addition of CO_2 to a gas stream of N_2, which was continuously supplied at a rate of 200 mL min^{-1} (±20) in order to mix the suspension and prevent oxygen accumulation. The flow rate was increased to 350 mL min^{-1} to prevent settling of the biomass for the wild-type in the event of floc formation. The photon flux density (I_{ph}, μmol photons m^{-2} s^{-1}) was measured with a LI-COR 190-SA 2π PAR (400–700 nm) quantum sensor (LI-COR, USA). The incident light intensity was measured at 28 points evenly distributed over the light-exposed surface of the front glass panel of the culture chamber (see Table 3 of Appendix 2 for light distribution). The light measurement was repeated for every experiment, and by averaging the 28 values, the surface-averaged incident light intensity was obtained $I_{ph,in}$. The outgoing light intensity $I_{ph,out}$ was measured in the same manner at the back side of the reactor. $I_{ph,out}$ was measured during the experiment because the transmission of light is dependent on the biomass concentration and pigmentation of the cells.

The reactors were operated in turbidostat mode to ensure a constant light regime. The reactors were equipped with a planar photodiode with integrated infrared filter (type SLD-70BG2, Silonex, Canada) behind the rear glass panel to measure the transmission of light through the culture. When light transmission decreased below the selected set-point, the Algaemist dilution pump (type 400A1, Watson Marlow, UK) was automatically engaged, and the culture was diluted with fresh medium. Each reactor was connected to a harvest vessel via an overflow. Foam formation was prevented by supplying a heat-sterilized 2 % (*v*/*v*) antifoam solution (Antifoam B, Mallinckrodt Baker B.V., the Netherlands) which was automatically pumped into the reactor for 1–2 min every 2 h using a time switch. The flow rate (1 to 1.75 mL min^{-1}) was adjusted manually depending on the amount of foam that had formed.

Photobioreactor operation

Reactors were heat-sterilized (90 min at 121 °C), and cultures were first grown in batch mode. The light intensity was increased stepwise as the biomass concentration increased. After the desired biomass concentration was achieved, and turbidostat control was initiated, cultures were allowed to adapt to the cultivation conditions for at least 36 h. Utilizing turbidostat control, the biomass concentration was maintained at a constant value by automatically diluting the reactor with fresh medium. The biomass concentration and the diluted volume were measured on a daily basis. The average areal biomass productivity was calculated from measurements performed at least 96 h following the adaptation phase. It was ensured that the cultivation was stable during this period and that the biomass concentration, dilution rate, and areal biomass productivity were

Table 1 Overview of the genetic properties of different antenna size mutants

Strain	Mutation method	Gene involved	Cell wall	References
BF4	UV mutagenesis	Unknown	+	(De Vitry and Wollman 1988; Olive et al. 1981)
Stm3LR3	RNA interference	Genes encoding for LHCI, LHCII, CP26 and CP29	−	(Mussgnug et al. 2007)
TLA2	Insertional mutagenesis	TLA2-CpFTSY	+	(Kirst et al. 2012)
AS2.2	Insertional mutagenesis	Unknown	+	(Bonente et al. 2011)

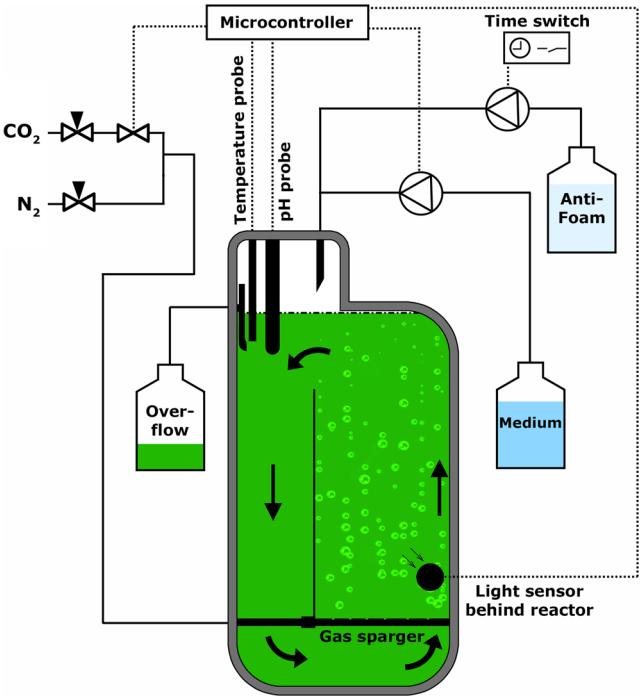

Fig. 1 Schematic overview of the experimental setup

constant. Every 24 h, the culture broth harvested in the overflow vessel was weighed and used for further analyses. The equation to calculate biomass productivity can be derived from the biomass balance and the definition of bioreactor productivity r_x (in g m^{-2} h^{-1}) as follows:

$$\frac{d(M_{x,R})}{dt} = \mu \cdot C_{x,R} \cdot V_R - F_{out} \cdot C_{x,out} \quad (1)$$

$$r_x = \mu \cdot C_{x,R} \quad (2)$$

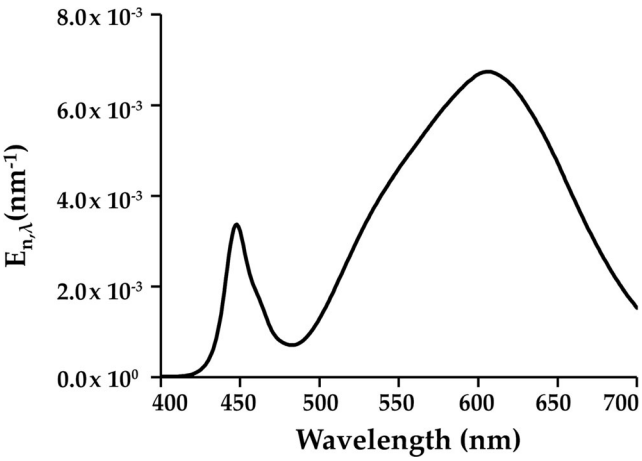

Fig. 2 Emission spectrum of the warm-white LEDs (BXRA W1200, Bridgelux, USA). The parameter $E_{n,\lambda}$ represents the relative fraction of PAR photons present within a 1-nm wavelength interval

Where $M_{x,R}$ is the biomass dry weight content (g) in the reactor, $C_{x,R}$ is the biomass dry weight concentration in the reactor (g L^{-1}), V_R is the reactor volume (L), F_{out} is the harvest flow collected in the overflow vessel (L h^{-1}), and $C_{x,out}$ is the biomass dry weight concentration of the harvest flow (g L^{-1}). By assuming no biomass accumulation ($dM_{x,R}/dt=0$), Eq. 3 is obtained.

$$0 = r_x \cdot V_R - F_{out} \cdot C_{x,out} \Leftrightarrow r_x = \frac{F_{out} \cdot C_{x,out}}{V_R} \quad (3)$$

Similarly, it can be deduced from the same biomass balance that the specific growth rate μ equals the reactor dilution rate D when assuming the reactor volume V_R is constant, and the reactor is ideally mixed ($C_{x,R}=C_{x,out}$):

$$\mu = D \quad (4)$$

Photobioreactor productivity is most effectively evaluated by comparing the areal biomass productivity (in g m^{-2} h^{-1}) in which the area reflects the illuminated area (A_R) of the photobioreactor, and accordingly:

$$r_x = \frac{F_{out} \cdot C_{x,out}}{A_R} \quad (5)$$

The biomass yield on light energy was calculated using the following equations:

$$Y_{x/ph} = \frac{r_x}{r_{ph}} \quad (6)$$

$$r_{ph} = \left(I_{ph,\ in} - I_{ph,\ out}\right) \cdot 1 \cdot 10^{-6} \quad (7)$$

Where r_{ph} is the areal light absorption rate (mol_{ph} m^{-2} h^{-1}). To calculate $Y_{x/ph}$ on an incident light energy basis, $I_{ph,out}$ is taken to be zero in Eq. 7.

Analyses

Biomass dry weight concentration To determine the biomass dry weight content, the culture broth was passed through glass fiber filters as described by Kliphuis et al. (2012) and, subsequently, the mass difference between the dried empty filters and the dried filters with algae was recorded. All measurements were performed in triplicate.

Biomass dry weight-specific optical cross section Light absorption was measured in a double-beam spectrophotometer (UV-2600, Shimadzu, Japan) equipped with an integrating

sphere (ISR-2600). With this setup, the effect of light scattering on the optical cross section is minimized since forward scatter is collected by the integrating sphere. The light intensity of the two beams following different paths is simultaneously compared. One light path comprised the reference sample (fresh medium) and the other contained the sample of the microalgae suspension. Absorbance was measured from 350 to 750 nm. The average absorbance that was measured between 740 and 750 nm was subtracted from the absorbance of 350 to 750 nm which is primarily induced by residual backward and sideward scatter (Dubinsky et al. 1986). Samples were withdrawn directly from the reactor, diluted 1:1 with fresh medium, and transferred to cuvettes (100.099-OS, Hellma, Germany) with a short light path of 2 mm. The wavelength-dependent dry weight optical cross section $a_{x,\lambda}$ ($m^2\ g^{-1}$) was calculated using Eq. 8.

$$a_{x,\lambda} = \frac{ABS_\lambda \cdot \ln 10}{C_x \cdot l} \tag{8}$$

Where ABS_λ is the absorbance measured at wavelength λ, ln(10) is the conversion factor to convert a base 10 logarithm to the natural logarithm, C_x is the biomass concentration ($g\ m^{-3}$) in the cuvette, and l is the cuvette thickness (m).

Cell number and cell size Cell number and cell size were determined with a Beckman Coulter Multisizer 3 (50 μm orifice, Beckman Coulter, USA). The samples were diluted with Isoton II dilution buffer. The biomass volume-averaged diameter ($d_{V,av}$, μm) of the cells was calculated according to the following equation:

$$d_{V,av} = \sum_{i=0}^{i=N} \frac{V_{p,i}}{V_p} \cdot d_{p,i} \tag{9}$$

In this equation, d_p is the average particle diameter at the interval $[i, i+1]$, $V_{p,i}$ is the biomass volume at the interval $[i, i+1]$, and Vp is the total biomass volume measured at the interval $[0, N]$. N is the total number of measurements.

Results

To estimate the potential of antenna size mutants, we constructed a simple mathematical microalgae growth model whereby we calculated the theoretical improvement in photobioreactor productivity by employing antenna size mutants. During long-term experiments under simulated mass culture conditions, we measured the areal biomass productivity (r_x) of four different antenna size mutants and two wild-type reference strains. In this context, mass culture conditions are defined as sunlight intensity (1500 μmol photons $m^{-2}\ s^{-1}$) and a biomass concentration (C_x) high enough to absorb 93 to 99 % of the incoming light energy, depending on the expected optimal value for each strain. For the exact values of light intensity and obtained experimental data of each experiment, please refer to Tables S1 to S5 of the supplementary material.

Model estimation of biomass productivity and light use efficiency

Using a simple kinetic model, we predicted the biomass specific growth rate (μ) and the biomass yield on light energy ($Y_{x/ph}$) for both an antenna size mutant and the wild-type strain as a function of the light intensity (I_{ph}). The mutant productivity and yield were estimated using the optical cross section of the BF4 mutant, which in our experiments had the lowest optical cross section of the tested mutants. For a detailed description of the model calculations, please refer to Appendix 1.

As shown in Fig. 3, at non-saturating light intensities, the specific growth rate μ of an antenna size mutant was predicted to be lower compared to the wild-type because the mutant has less light absorption capacity, limiting the amount of energy available for growth. Antenna size mutants are not expected to have a higher maximum biomass specific growth rate than the wild-type strain. As clearly depicted in Fig. 3, the potential of antenna size mutants can be found in their increased light use efficiency at high light intensities compared to the wild-type strain. By absorbing less light, the cell-specific light uptake is reduced to a rate that can continue to be processed efficiently by photosynthesis.

The filled area below the $Y_{x/ph}$–I_{ph} curve represents the maximal biomass productivity per unit of illuminated surface area (r_x, $g\ m^{-2}\ h^{-1}$). The incident light intensity is 1500 μmol photons $m^{-2}\ s^{-1}$, and the light intensity at the back side of the photobioreactor is assumed to be equal to the compensation point $I_{p,c}$ of photosynthesis, where net growth is zero. At this point, the $Y_{x/ph}$–I_{ph} curve crosses the x-axis. The model then predicts an r_x of 1.7 $g\ m^{-2}\ h^{-1}$ for the wild-type strain versus 3.7 $g\ m^{-2}\ h^{-1}$ for the antenna mutant. Otherwise stated, based on our model estimation, we conjecture a doubling of photobioreactor productivity employing the mutant when compared to the wild-type.

Optical cross section of wild-type and antenna size mutants

The antenna size mutants and the wild-type strain were grown in photobioreactors in turbidostat mode as described in the "Materials and methods" section. After it was established that C_x and D were constant and the cultures were completely acclimated to the applied light regime, the biomass dry weight-specific optical cross section (a_x) was measured. The absorption spectra of the wild-type and the mutants, which are

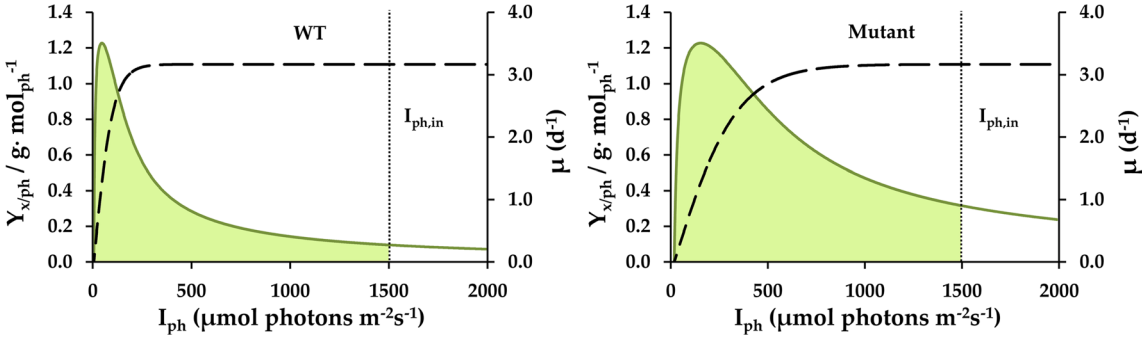

Fig. 3 Estimation of the biomass specific growth rate (μ, *dashed line*) and biomass yield on light energy ($Y_{x/ph}$, *solid line*) of the wild-type strain and an antenna size mutant as a function of the light intensity (I_{ph}). The mutant performance was estimated using the optical cross section of the BF4 mutant. By numerical integration of the area below the $Y_{x/ph}$–I_{ph} curves, the biomass productivity per illuminated surface area (r_x) is obtained. The *dotted line* indicates the incident light intensity used for the experiments in this study

normalized to dry biomass weight, were measured to compare the degree of pigmentation (Fig. 4.). A lower optical cross section corresponds to more transparent cells and thus a more prominent mutant phenotype. It was confirmed that the mutations were successful since all mutants absorbed less light per unit of dry biomass than the wild-type strain. The BF4 mutant demonstrated the lowest pigmentation.

Areal biomass productivity of wild-type strain

The areal productivity of the wild-type strains was measured at an incident light intensity ($I_{ph,in}$) of 1500 μmol photons m^{-2} s^{-1} in eight different continuous experiments to provide a robust dataset for comparison with the mutants. The measured areal biomass productivity for the wild-type strain CC1690 was 1.9 g m^{-2} h^{-1}, the biomass concentration in the photobioreactor was 2.6 g L^{-1}, and the biomass specific growth rate was 1.3 day^{-1}. As illustrated in Fig. 5, there was variability in the measured productivities. In four of the eight experiments, the areal productivity r_x clustered around 1.9 g m^{-2} h^{-1} and, in the other four experiments, r_x clustered around 2.4 g m^{-2} h^{-1}. The latter value correlated with the formation of cell agglomerates in four of the eight wild-type experiments, which led to increased productivity values. Since single *Chlamydomonas* cells featured a size of approximately 8 μm during all experiments, cultures expressing agglomerate formation could be clearly discerned. In the other four experiments with the wild-type, no agglomeration was detected. The mutant strains at no time exhibited agglomerate formation. To exclude agglomerate formation as a factor influencing productivity, only wild-type experiments without agglomerate formation were compared to the mutant experiments. The averaged results of these four experiments are indicated in Fig. 6.

In order to demonstrate the magnitude of oversaturation of the wild-type strain at an $I_{ph,in}$ of 1500 μmol photons m^{-2} s^{-1}, this strain was also cultivated at an $I_{ph,in}$ of 850 μmol photons m^{-2} s^{-1}. At 1500 μmol photons m^{-2} s^{-1}, r_x was ascertained to be 1.9 versus 1.5 g m^{-2} h^{-1} at 850 μmol photons m^{-2} s^{-1} (Fig. 6). C_x was found to be substantially higher at 1500 μmol photons m^{-2} s^{-1} (2.6 g L^{-1}) than at 850 μmol photons m^{-2} s^{-1} (1.9 g L^{-1}) because additional biomass is required to absorb the additional light. The dilution rate (D), which is equal to the biomass specific growth rate (μ), did not increase at an $I_{ph,in}$ of 1500 μmol photons m^{-2} s^{-1} because the higher light intensity was compensated by a higher biomass concentration. The

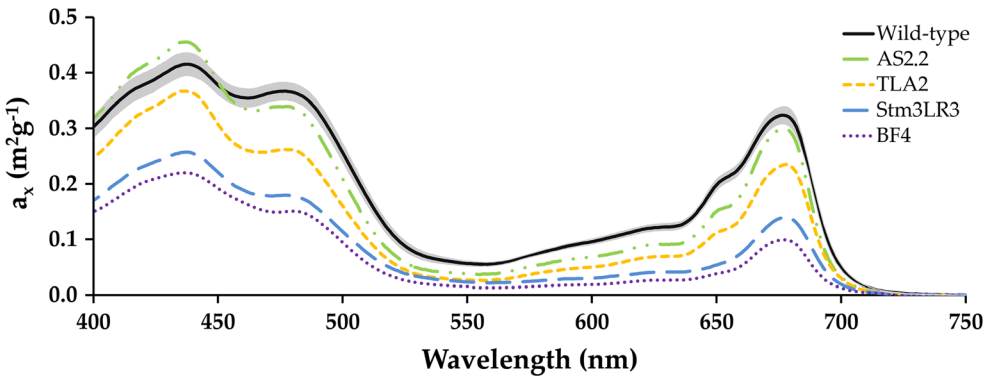

Fig. 4 Wavelength-dependent biomass dry weight-specific optical cross section of four mutant strains and wild-type. *Lines* represent the average of four reactor experiments±SD (wild-type) and a single reactor experiment (mutants). The incident light intensity ($I_{ph,in}$) was 1500 μmol photons m^{-2} s^{-1}. The culture outgoing light intensity ($I_{ph,out}$) was 10 μmol photons m^{-2} s^{-1} for the wild-type and 50 μmol photons m^{-2} s^{-1} for the mutants

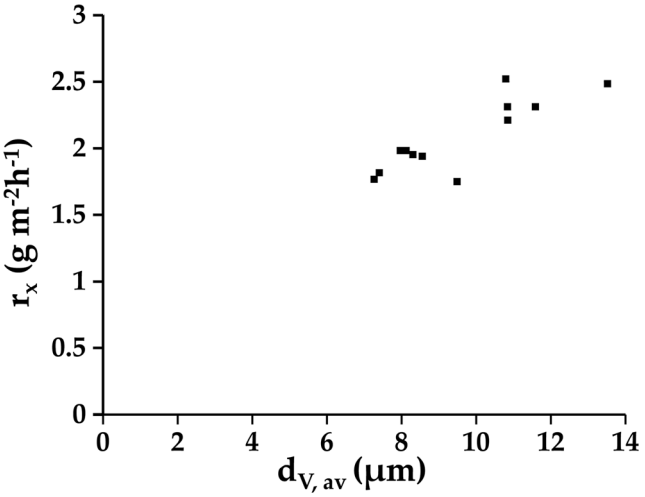

Fig. 5 Areal biomass productivity (r_x) of wild-type strain CC1690 as a function of the volume-averaged diameter ($d_{V,av}$) of the cells and agglomerates of cells. Each data point originates from a 24-h interval measurement during which $d_{V,av}$ and r_x were determined

biomass yield on light energy decreased from 0.51 g mol_{ph}^{-1} at 850 μmol photons m^{-2} s^{-1} to 0.37 g mol_{ph}^{-1} at 1500 μmol photons m^{-2} s^{-1}, demonstrating the effect of photosaturation.

Areal biomass productivity of antenna size mutants

The performance of four *Chlamydomonas* antenna size mutants was compared to that of wild-type strain CC1690 at an $I_{ph,in}$ of 1500 μmol photons m^{-2} s^{-1}. None of the antenna mutants exhibited the anticipated increase in areal biomass productivity. The best performing mutants achieved similar r_x values to that of the wild-type (Figs. 7 and 8a). We also

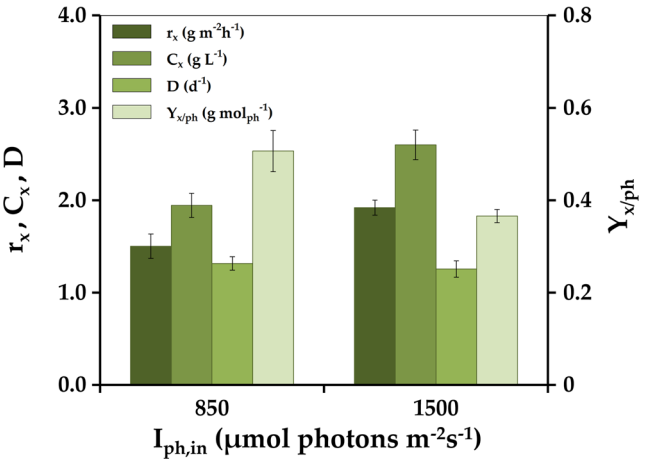

Fig. 6 Areal biomass productivity (r_x), dry biomass concentration (C_x), dilution rate (D), and biomass yield on absorbed light ($Y_{x/ph}$) of wild-type at medium and high light conditions. Each *bar* represents the average of $n \geq 4$ (1500 μmol photons m^{-2} s^{-1}) or $n \geq 5$ (850 μmol photons m^{-2} s^{-1}) data points±pooled SD that were measured on a daily basis within four (1500 μmol photons m^{-2} s^{-1}) or two (850 μmol photons m^{-2} s^{-1}) reactor experiments

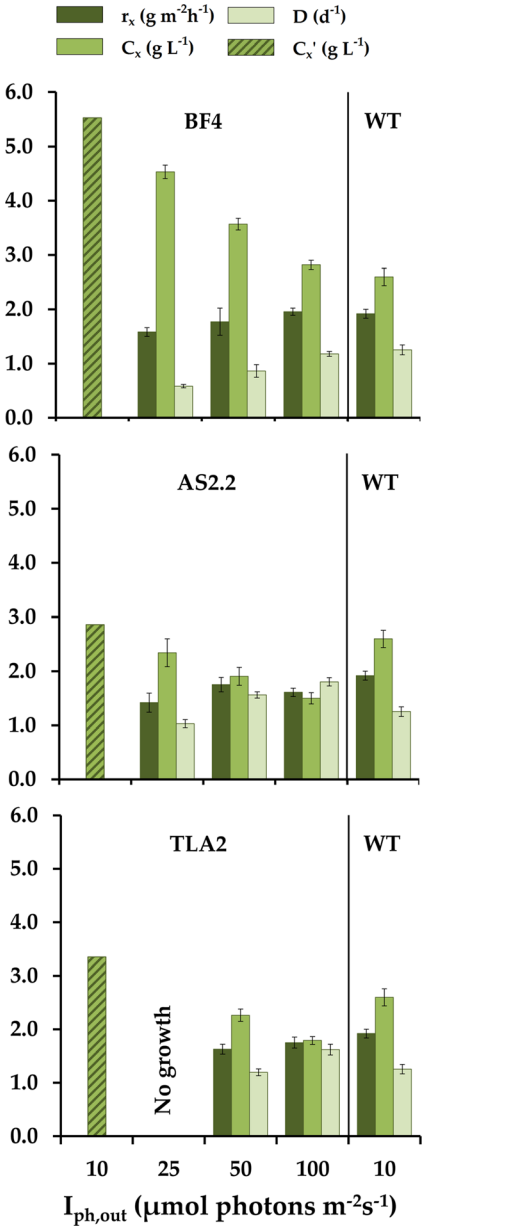

Fig. 7 Areal biomass productivity (r_x), biomass concentration (C_x), and dilution rate (D) at an $I_{ph,in}$ of 1500 μmol photons m^{-2} s^{-1} and different $I_{ph,out}$. C_x' is the extrapolated biomass concentration of a mutant strain if it would have been cultivated at the same $I_{ph,out}$ (10 μmol photons m^{-2} s^{-1}) as the wild-type. For mutants, each *bar* represents the average of $n \geq 4$ data points±SD that were measured on a daily basis within one reactor experiment. For wild-type, the *bars* represent the average of $n=20$ data points±pooled SD that were measured on a daily basis within four different reactor experiments

investigated the effect of the outgoing light intensity on r_x. For two of the mutants (BF4 and TLA2), r_x was significantly higher at an $I_{ph,out}$ of 100 μmol photons m^{-2} s^{-1} than at 25 μmol photons m^{-2} s^{-1}.

At an $I_{ph,out}$ of 25 μmol photons m^{-2} s^{-1}, the BF4 mutant, which has the lowest optical cross section (a_x) of all mutants (Fig. 4), attained the highest biomass concentration (4.5 g L^{-1})

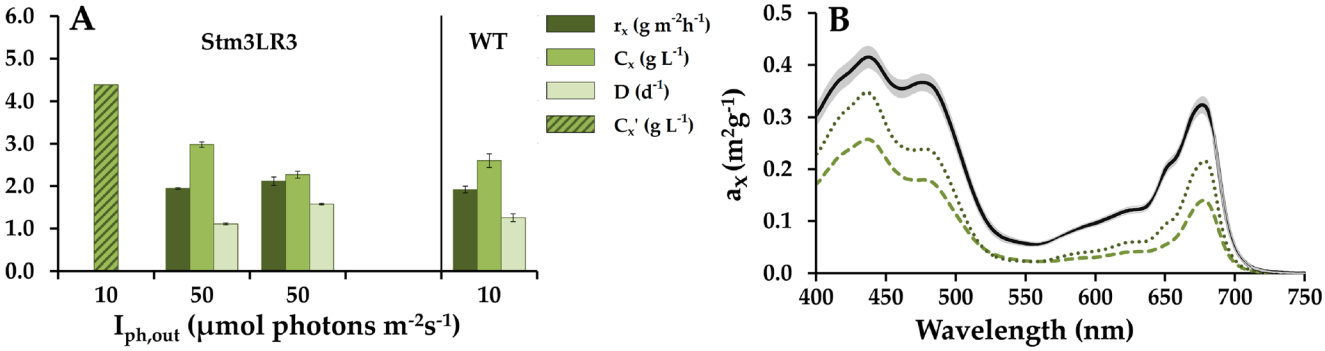

Fig. 8 **a** Areal biomass productivity (r_x) at various outgoing light intensities ($I_{ph,out}$). For Stm3LR3, each *bar* represents the average of $n \geq 4$ data points±SD that were measured on a daily basis within one reactor experiment. For the wild-type, see Fig. 7. **b** Wavelength-dependent biomass dry weight-specific optical cross section (a_x) of the Stm3LR3 culture with a C_x of 3.0 g L^{-1} (*dashed line*) and with a C_x of 2.3 g L^{-1} (*dotted line*), as shown in (**a**). *Lines* represent the average of four reactor experiments±SD (wild-type) and a single reactor experiment (Stm3LR3)

measured in this series of experiments. In contrast, at an outgoing light intensity of 10 µmol photons m^{-2} s^{-1}, wild-type biomass concentration was only 2.6 g L^{-1}. Although the BF4 biomass concentration was much higher than that of the wild-type, its specific growth rate µ was markedly lower, resulting in a lower areal biomass productivity than that of the wild-type.

In all mutant cultures, a higher outgoing light intensity (i.e., a lower biomass concentration) resulted in a higher biomass specific growth rate as more light was available per cell. At an outgoing light intensity of 100 µmol photons m^{-2} s^{-1}, AS2.2 and TLA2 attained even higher specific growth rates than the wild-type. However, this was counterbalanced by a much lower biomass concentration and thus a lower areal productivity than the wild-type.

We attempted to cultivate BF4 and TLA2 at an $I_{ph,out}$ of 10 µmol photons m^{-2} s^{-1}, but these cultures collapsed. The cultures were first grown in batch in order to attain the biomass concentration corresponding to $I_{ph,out}$=10 µmol photons m^{-2} s^{-1}. However, this biomass concentration could not be achieved as growth ceased at higher biomass densities. TLA2 exhibited the same behavior at $I_{ph,out}$=25 µmol photons m^{-2} s^{-1}.

To illustrate the effect of pigment reduction on the biomass concentration in a turbidostat-controlled photobioreactor with a fixed $I_{ph,in}$ and $I_{ph,out}$, we extrapolated the biomass concentration of the mutants to the same $I_{ph,out}$ (10 µmol photons m^{-2} s^{-1}) that was used for the wild-type strain. The extrapolated biomass concentration is depicted as C_x' in Figs. 7 and 8a. Please refer to Appendix 3 for the calculation of C_x'. The following extrapolated biomass concentrations were calculated: BF4, 5.5 g L^{-1}; TLA2, 3.4 g L^{-1}; AS2.2, 2.9 g L^{-1}; and Stm3LR3, 4.4 g L^{-1}. Wild-type C_x was 2.6 g L^{-1}.

Several experiments have been performed with cell wall-less antenna size mutants (TLA1 CW- (Polle et al. 2003) and Stm3LR3 (Mussgnug et al. 2007)) because cell wall removal was required in the process of creating these mutants. However, cultivation was cumbersome. Cell wall-less mutants were found to be fragile in a well-mixed photobioreactor. This fragility also created difficulty when determining the biomass dry weight concentration utilizing vacuum filtration. Due to cell breakage, less biomass was retained on the fiber filters (i.e., biomass was lost), and there was greater variation in the dry weight measurement. However, for the cell wall-less mutant Stm3LR3, we managed to perform two successful experiments by minimizing the hydrodynamic forces in the photobioreactor (i.e., a lower gas flow). Figure 8a illustrates the results of these experiments, which are similar to the results established for the wild-type and the other mutants. The measured biomass concentrations and dilution rates varied significantly for the two replicate experiments; the analysis of the accompanied absorption spectra revealed an increased pigmentation for the culture with the lowest biomass concentration (Fig. 8b). The productivity was not markedly affected by the shift in pigmentation.

In Fig. 9, the performance of the strains is depicted as the biomass yield on light energy ($Y_{x/ph}$). For all mutant strains that were cultivated under different $I_{ph,out}$, only the most significant measured yield is indicated. $Y_{x/ph}$ can be expressed based on incident light or on absorbed light, the latter being corrected for the outgoing light intensity (see Eqs. 6 and 7). In the latter case, strain performance is slightly higher than when expressed on an incident light basis, especially for mutants cultivated at a high $I_{ph,out}$. If a mutant showed a similar r_x but higher $I_{ph,out}$ (e.g., 100 µmol photons m^{-2} s^{-1} vs 10 µmol photons m^{-2} s^{-1} non-absorbed light) compared to the wild-type strain, its $Y_{x/ph}$ on an absorbed light basis will be higher since less light was absorbed to achieve the same productivity.

An important observation was that all mutant cultures were ascertained to be more sensitive to photodamage than the wild-type. Growth of the mutants sometimes ceased when the light intensity was increased too much. The same increase in light intensity did not harm the wild-type strain. For the mutants, this resulted in very low dilution rates or no dilution

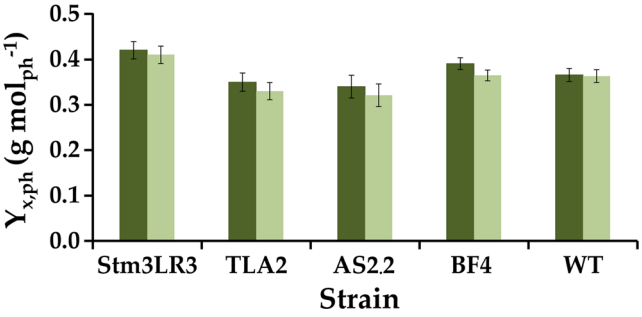

Fig. 9 Biomass yields on light energy ($Y_{x,ph}$) of antenna size mutants and the wild-type. $Y_{x,ph}$ is indicated on an incident light energy basis (*light bars*) and on an absorbed light energy basis (*dark bars*). For the mutants, each *bar* represents the average of $n \geq 4$ data points±SD that were measured on a daily basis within one reactor experiment. For the wild-type, the *bars* represent the average of $n=20$ data points±pooled SD that were measured on a daily basis within four reactor experiments. For results based on absorbed light energy, the outgoing light intensity for each strain was Stm3LR3, 50 μmol photons m^{-2} s^{-1}; TLA2, 100 μmol photons m^{-2} s^{-1}; AS2, 50 μmol photons m^{-2} s^{-1}; BF4, 100 μmol photons m^{-2} s^{-1}; and WT, 10 μmol photons m^{-2} s^{-1}

at all. These cultures often recovered when the light intensity was reduced; however, in some cases, the culture collapsed while the wild-type strain cultivated under identical conditions did not exhibit any sign of stress.

In additional experiments, we compared the performance of three mutants with the wild-type at an $I_{ph,in}$ of 850 μmol photons m^{-2} s^{-1} and an $I_{ph,out}$ of 50 μmol photons m^{-2} s^{-1} for the mutants and 10 μmol photons m^{-2} s^{-1} for the wild-type strain (Fig. 10). Mutants did not exhibit a higher r_x than the wild-type (1.5 g m^{-2} h^{-1}) but attained similar values (1.2 to 1.4 g m^{-2} h^{-1}).

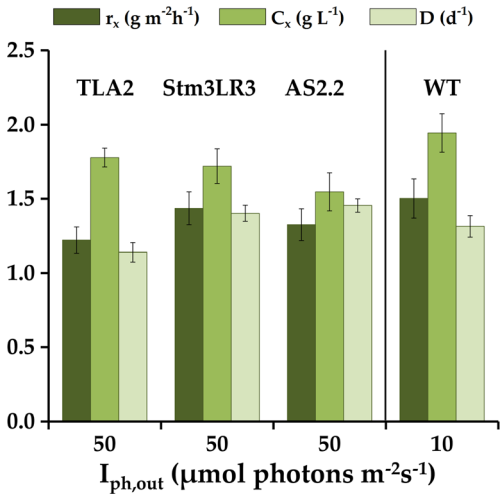

Fig. 10 Areal biomass productivity at an ingoing light intensity of 850 μmol photons m^{-2} s^{-1} and an outgoing light intensity of 50 photons μmol photons m^{-2} s^{-1} for the mutants and 10 μmol photons m^{-2} s^{-1} for wild-type . For the mutants, the *error bars* indicate the standard deviation ($n \geq 5$) of a single reactor experiment. For the wild-type, the *bars* represent the average of $n=11$ data points±pooled SD that were measured on a daily basis within two reactor experiments

Discussion

Wild-type *Chlamydomonas* cells are easily oversaturated by high light intensities. We measured biomass yields on light ($Y_{x/ph}$) of 0.37 g mol_{ph}^{-1} at an $I_{ph,in}$ of 1500 μmol photons m^{-2} s^{-1} and 0.51 g mol_{ph}^{-1} at an $I_{ph,in}$ of 850 μmol photons m^{-2} s^{-1}. A biomass yield on light energy of 1.25 g mol_{ph}^{-1} can be attained at a very low incident light intensity (Kliphuis et al. 2012) when photosynthesis is limited by light and heat dissipation is minimal or absent. Theoretically, the difference between this value and the yields we measured determines the scope for improvement of the biomass productivity employing genetically engineered strains with truncated antenna complexes. By preventing energy losses resulting from the oversaturation of the photosystems, the excess light energy can potentially be converted to algal biomass, increasing the areal biomass productivity of the culture.

The optimal biomass concentration to maximize biomass productivity is found when, at the back of the reactor, the net photosynthesis rate is equal to zero (Takache et al. 2010). The light intensity at this condition is the photosynthetic compensation point. For *C. reinhardtii*, the photosynthetic compensation point was found to be approximately 10 μmol photons m^{-2} s^{-1} (Takache et al. 2010; Vejrazka et al. 2013). This value, however, does not represent a sharp optimum. During the experiments with the wild-type cells described in this paper, an outgoing light intensity of 10 μmol photons m^{-2} s^{-1} was assumed to result in the highest productivity. Since antenna size mutants have less light absorption capacity, their photosynthetic compensation point will be higher than that of the wild-type. For this reason and with no reference data available, maximal mutant productivity was sought for in a range of outgoing light intensities of 25–100 μmol photons m^{-2} s^{-1}.

We used our model to estimate the areal biomass productivity of cells with a high (wild-type) or low (antenna size mutants) pigment content under mass culture conditions. The predicted r_x for the wild-type strain (1.7 g m^{-2} h^{-1}) is very much in accordance with the experimentally achieved r_x (1.9 g m^{-2} h^{-1}). In contrast to the expectations, the experimentally attained results for the mutants did not meet the model predictions. Two important assumptions were made that could have led to an overestimation of the areal biomass productivity. Foremost, a_x is presupposed to be constant at any position in the photobioreactor while, in reality, the spectrum at the back of the reactor primarily consists of green light, which can hardly be absorbed by green algae and therefore results in a lower photosynthetic rate at that specific position of the reactor. The second assumption is that there is no backscattering in the

photobioreactor. Otherwise stated, all photons are absorbed by the culture except for the minimal amount of light leaving the reactor at the back.

Overall, mutant cultures did not exhibit the estimated doubling in productivity in comparison to the wild-type cultures. Mutant cultures contained more cell debris than the wild-type culture, probably as a consequence of a higher cell death rate. This could also explain why mutant cultures displayed a greater tendency to foam and were easily overgrown by bacteria. Microscopic analysis revealed that a small fraction of the mutant cells was colorless, most plausibly the result of severe photodamage. During the cultivations, both biomass concentration and specific growth rate fluctuated more than that of the wild-type, and mutants were more sensitive to abrupt light shifts than the wild-type strain.

For three of the mutants, the areal biomass productivity increased at an increasing higher outgoing light intensity (from 25 to 100 μmol photons m^{-2} s^{-1}). This signifies that antenna size mutants do, indeed, require more light to achieve the same photosynthetic activity as the wild-type, for which the optimal $I_{ph,out}$ was estimated to be only 10 μmol photons m^{-2} s^{-1}. In some cases, the applied outgoing light intensities may have been too low for cells with a low pigment content, which explains why mutants could not be cultivated at an outgoing light intensity of 10 μmol photons m^{-2} s^{-1}. We hypothesize that, under the resulting light regime, respiration became a significant factor balancing the photosynthetic rate. Stated differently, the antenna size mutants probably experienced a dark zone at the back of the reactor in which the net photosynthetic rate was negative, and this even led to the absence of net growth in the photobioreactor at very low levels of $I_{ph,out}$ (<10 μmol photons m^{-2} s^{-1}). However, even with higher outgoing light intensities, cultivation of mutants did not fulfill the expectation of improved biomass productivity.

For the cell wall-less mutants, it is plausible that shear stress caused by hydrodynamic forces in the photobioreactor resulted in breakage of the cells, an increase in the amount of cell debris, which subsequently led to an uncontrolled growth of bacteria. Unstable cultures and unreliable biomass concentrations evidenced that cell wall-less mutants were unsuitable for our experimental conditions. A remarkable finding was that, under the same light regime in two reactor experiments, different biomass concentrations were attained for the Stm3LR3 mutant. This can only be explained by an increased optical cross section of the culture. Otherwise stated, this mutant possibly lost part of its phenotype after the first reactor experiment was performed.

It is often erroneously supposed that the increased light penetration in antenna size mutant cultures is a major factor for improving the biomass productivity. However, in order to maximize productivity under mass culture conditions, light penetration is a parameter that is automatically controlled by adjusting the biomass concentration. This results in a photobioreactor with a similar light gradient as the wild-type culture but with a higher biomass concentration. Mutants could not, however, be cultivated in the outgoing light intensity assumed to be optimal for the wild-type strain. The photosynthetic compensation point of mutants appears to be higher than that of the wild-type strain. This statement is substantiated by our experimental data. An increased light transmittance (i.e., by applying a higher outgoing light intensity) in an antenna size mutant culture was ascertained to be a requirement for maximizing productivity and not a feature of antenna size mutants with a higher transparency.

The antenna size mutants were found to absorb less light per cell and, as a consequence, could be cultivated at higher biomass concentrations than the wild-type depending upon the actual optical cross section of the antenna size mutant and the selected $I_{ph,out}$. In the event of a healthy culture, the lower light absorption rate per cell should have resulted in a lower dissipation rate and, therefore, a higher biomass productivity and biomass yield on light energy. We did not observe an increased biomass yield on light energy. The attained results suggest that the genetic modifications resulted in complications that ultimately eliminated the anticipated beneficial effect of a truncated antenna complex. Therefore, a possible explanation for the reduced fitness of the mutants during long-term experiments is that the modified antenna complexes lost essential regulatory functions involved in the distribution, conversion, or dissipation of light energy.

Most antenna size mutants are deficient in chlorophyll a/b binding proteins and the subunits necessary for assembling the peripheral components of the light-harvesting antenna. However, many of these binding proteins and subunits have been evidenced to play a role in photoprotection mechanisms and energy distribution. Loss of CP29, a binding protein, leads to impaired state transitions (Tokutsu et al. 2009). LHCSR3, a Chl a/b binding stress-related member of the LHC protein superfamily, is of high importance for cell survival at high light intensities as it is essential for the performance of heat dissipation (qE) (Tokutsu and Minagawa 2013). Cells survival after switching from a low to high light intensity has been found to be lower when LHCSR genes were lacking (Peers et al. 2009). Moreover, several antenna proteins are known to function in photoprotective mechanisms in microalgae and higher

plants (Alboresi et al. 2009). For example, recently, it was shown that the LHCII protein LHCBM9 is highly important for photoprotection during prolonged stress conditions in *C. reinhardtii* (Grewe et al. 2014). Antenna size mutants have also been generated in which binding proteins are not downregulated in a direct way (Perrine et al. 2012). In these mutants, the chlorophyll b formation by the enzyme chlorophyllide a oxygenase (CAO) is inhibited. It seems that this would be a more safe approach. However, there is certain evidence indicating that chlorophyll b functions as an antenna size regulator and possesses an additional important structural role in protein-pigment complexes (Tanaka and Tanaka 2000). This signifies that a reduction of Chl b would, in any case, be accompanied with a decrease in antennae proteins involved in photoprotection.

Another possible explanation for the reduced fitness observed in all tested mutants is the high probability of unintended side effects resulting from genetic engineering. Using insertional mutagenesis (TLA2 and AS2.2 mutant) for example, multiple DNA deletions or DNA rearrangements may take place, disturbing unknown gene functions. Also, the use of antibiotic resistance cassettes (AS2.2 and Stm3LR3 mutant) accompanied by accumulation of a protein conferring antibiotic resistance in the cell might affect cell fitness or maximal growth performance. In addition, random mutagenesis using UV irradiation (BF4 mutant) could also have affected multiple genes responsible for important metabolic processes. In another study, antenna size mutants generated based on this technique were found to show lower maximum specific growth rates and a poorer stability than the wild-type (Huesemann et al. 2009). Although the resulting mutants, tested in this study, were viable, it is not unlikely that extreme cultivation conditions like very high light intensities revealed their limitations.

A possible way to exclude unintended side effects is by complementation of the mutants with the gene responsible for the pigment reduction. The resulting strain(s) will have normal pigmentation but might still suffer from the genetic side effects. Such a reference strain should then be assessed for its productivity. In our study, we did not choose for this approach, first of all because generation and assessment of these complemented strains, for each mutant separately, would be very time consuming. Second, the goal of our study was to investigate whether existing, genetically engineered antenna mutant strains can outperform normally pigmented wild-type strains under mass culture conditions. In-depth analyses of the different mutant strains are required to find the cause of their reduced fitness, which is beyond the scope of this study.

Other researchers have encountered similar issues with antenna size mutants. Although *Cyclotella* sp. mutants demonstrated a higher light saturation level, cultivation was described as less stable than the wild-type, and mutant cultures were found to collapse within 10 days (Huesemann et al. 2009). Less resistance against high light intensities is provided as a possible explanation. In another study, antenna mutants of the cyanobacterium *Synechocystis* lacking the complete light-harvesting antenna were found to have a lower robustness and fitness than the wild-type (Kwon et al. 2013). This strain could not be cultivated at light intensities higher than 300 μmol photons m^{-2} s^{-1}, and proteomic analysis revealed an increased concentration of enzymes that are normally synthesized in stress situations. This result shows that, for cyanobacteria, at least some light-harvesting antenna subunits are essential for cell survival at high light intensities. Another mutant created by the same researchers which lacked only specific antenna subunits did not exhibit this stress-phenotype. However, these mutants had not been cultivated at a high light intensity where the need for photoprotection is highest and the effects of photosaturation are maximal. In a different study regarding a *Synechocystis* mutant, researchers describe phycobilisome reduction as a suboptimal strategy to improve culture productivity as the modifications lead to changes in many critical components that are essential for cell function which, consequently, results in decreased productivity (Liberton et al. 2013). Distinguishing essential photoprotection subunits from subunits that are strictly involved in light-harvesting and subsequently maintaining these essential subunits in the mutant selection procedure might be the crucial element to the realization of the ideal antenna size mutant (Bonente et al. 2011; Peers et al. 2009). The productivity increase achieved by genetically engineering *Chlamydomonas perigranulata* appears to be the only successful experiment so far that was conducted under mass cultivation conditions (Nakajima et al. 2001). The LHCI mutant demonstrated a 1.5 times higher productivity than the wild-type at an $I_{ph,in}$ of 2000 μmol photons m^{-2} s^{-1}. The applied light regime, however, is inadequately described, which raises the question whether the wild-type, being the negative control, was cultivated under optimal conditions. It would be interesting to study this specific mutant in more detail under well-defined light conditions in a turbidostat-controlled reactor.

During their generation, most antenna size mutant strains were selected based solely on their low pigment

content. Therefore, to prevent loss of photoprotection in the future, in addition to a low absorptive capacity, high light resistance of individual cells should be a criterion during the selection procedure. Screening for high light resistance is easily implemented and will lead to strains that are much more appropriate as starter cultures for further analysis of their biomass productivity under mass culture conditions. Being aware that algae have the natural capability to halve their pigment content at high light conditions, there must be ways to achieve this by genetic engineering without loss of photoprotection and without genetic side effects. The generation and evaluation of antenna size mutants is a process in which researchers from various disciplines are involved, each with their own focus and expertise. In consideration of this, we would like to emphasize that the engineering aspects of optimizing productivity of antenna size mutants should not be underestimated. Accurate and reliable productivity measurements under simulated mass culture conditions are essential for a viable evaluation of mutant performance. This requires knowledge of the optimal conditions to maximize productivity of microalgae cultures and how these conditions change in the event of pigment reduction. In addition, a continuous photobioreactor system combined with a well-defined light regime is of critical importance to create and maintain optimal cultivation conditions. By modeling microalgae productivity using experimental data from simulated mass culture conditions, a more solid base is obtained to describe the future potential and both theoretical and practical implications of antenna size reduction. In this perspective, it is advisable to involve bioprocess engineers in an early stage of antenna size mutant creation process as well. By combining expertise of molecular biology and bioprocess engineering, an improved understanding of the requirements for creating the most superior antenna size mutant will be obtained.

In conclusion, in this study, we present the areal biomass productivities of antenna size mutants quantified under simulated mass culture conditions. The long-term photobioreactor experiments under turbidostatic control combined with biomass dry weight measurements and a well-defined light regime provided a reliable basis to study culture productivity. A fixed incident light intensity of 1500 μmol photons m^{-2} s^{-1} guaranteed oversaturation of the wild-type strain and provided the conditions in which antenna size mutant cultures were anticipated to demonstrate a higher light use efficiency and doubling of productivity. The antenna mutants we tested were confirmed to have a lower light absorption capacity than the wild-type. The mutants, however, did not exhibit the expected increase in biomass productivity under mass culture conditions. The highest mutant productivity values were comparable (1.8–2.1 g m^{-2} h^{-1}) to that of wild-type productivity (1.9 g m^{-2} h^{-1}). The biomass yield on light energy ranged between 0.33 and 0.41 g mol_{ph}^{-1} for the mutants and was 0.36 g mol_{ph}^{-1} for the wild-type strain. Under turbidostat control, mutant cultures required a higher outgoing light intensity than the wild-type culture in order to achieve maximal areal productivity. Observations indicated a reduced fitness of the mutant cultures caused by a high light intensity. This might be explained by either a higher susceptibility to photodamage compared to the wild-type strain or by unintended side effects of the genetic modifications. The principle of antenna size reduction is a promising strategy to increase productivity. However, for future generations of antenna size mutants, in addition to reducing the light absorptive capacity of the cells, emphasis must be placed on tolerance to high light conditions that are evident in mass culture conditions.

Acknowledgements This work is part of the research programme of the Foundation for Fundamental Research on Matter (FOM) which is part of the Netherlands Organization for Scientific Research (NWO). This project was conducted within the research programme of BioSolar Cells, co-financed by the Dutch Ministry of Economic Affairs.

Appendix 1. Microalgae growth model and photobioreactor productivity

A simple kinetic model was constructed to describe microalgae productivity as a function of the light intensity. The model is based on two compartments. In the first compartment, the chloroplast, there is photosynthetic production of 3-carbon sugars (triose) symbolized by the 1-carbon sugar equivalent CH_2O. This sugar production rate in the chloroplast (q^c_{CH2O}, $mol_{CH2O} mol_x^{-1} s^{-1}$) is dependent upon light intensity I_{ph} and described by the hyperbolic tangent model of Jassby and Platt (1976):

$$q^c_{CH2O} = q^c_{CH2O,m} \cdot \tanh\left(\frac{I_{ph} \cdot a_x \cdot Y_{CH2O/ph,m}}{q^c_{CH2O,m}}\right) \quad \text{(A1)}$$

Where I_{ph} is the PAR photon flux density ($mol_{ph} m^{-2} s^{-1}$), $q^c_{CH2O,m}$ is the maximal sugar production rate ($mol_{CH2O} mol_x^{-1} s^{-1}$) in the chloroplast, $Y_{CH2O/ph,m}$ is the maximal yield of sugar on light energy ($mol_{CH2O} mol_{ph}^{-1}$), and a_x is the spectrally averaged optical cross section of the microalgae

Table 2 Overview of the model parameters

Parameter	Value	Unit	Description	References
μ_m	0.132	h^{-1}	Maximal biomass specific growth rate; assumed to be equal for WT and BF4	(Janssen et al. 2000)
$q^c_{CH2O,m}$	$6.17 \cdot 10^{-5}$	$mol_{CH2O} mol_x^{-1} s^{-1}$	Maximal 3-carbon sugar production rate; calculated from μ_m using Eq. A4.	
m_{CH2O}	$3.5 \cdot 10^{-6}$	$mol_{CH2O} mol_x^{-1} s^{-1}$	Biomass specific maintenance rate	(Kliphuis et al. 2012)
$Y_{x/CH2O}$	0.63	$mol_x mol_{CH2O}^{-1}$	Biomass yield on 3-carbon sugar using ammonia as N-source	(Kliphuis et al. 2012)
a_x (WT)	6.2	$m^2\ mol_x^{-1}$	Spectrally averaged optical cross section	This paper
a_x (Mutant)	1.9	$m^2\ mol_x^{-1}$	Spectrally averaged optical cross section	This paper
$Y_{CH2O/ph,m}$	0.10	$mol_{CH2O} mol_{ph}^{-1}$	Maximal yield of 3-carbon sugar on light energy	(Blanken et al. 2013)
M_x	24	$g\ mol_x^{-1}$	Biomass dry weight to C-mol conversion factor for algae	(Duboc et al. 1999)

($m^2\ mol_x^{-1}$). a_x can be calculated according to the following equation:

$$a_x = \sum_{\lambda=400}^{\lambda=700} a_{x,\lambda} \cdot E_{n,\lambda} \cdot \Delta\lambda \quad \text{(A2)}$$

In which $a_{x,\lambda}$ is the optical cross section at wavelength λ. $E_{n,\lambda}$ (nm^{-1}) represents the normalized spectral distribution of the light source (Fig. 2). It is the fraction of photons in the PAR region in a 1-nm interval at specific λ.

The cell minus the chloroplast comprises the second compartment in which the 3-carbon sugar is used to build new biomass at a specific growth rate μ. Another part of the sugar is respired in the mitochondria to provide energy (ATP) to support the growth reactions and to fulfill the maintenance requirements. The consumption of sugar in the chloroplast can be described by Pirt's Law (Pirt 1965), resulting in the following relation:

$$\mu = \left(q^c_{CH2O} - m_{CH2O}\right) \cdot Y_{x/CH2O} \quad \text{(A3)}$$

and:

$$\mu_m = \left(q^c_{CH2O,m} - m_{CH2O}\right) \cdot Y_{x/CH2O} \quad \text{(A4)}$$

In these equations, m_{CH2O} is the biomass specific maintenance rate ($mol_{CH2O} mol_x^{-1} s^{-1}$), $Y_{x/CH2O}$ is the biomass yield on 3-carbon sugar ($mol_x mol_{CH2O}^{-1}$), and μ_m is the biomass specific growth rate (s^{-1}).

The biomass on yield on light energy ($mol_x mol_{ph}^{-1}$) can now be calculated as follows:

$$Y_{x/ph} = \frac{\mu}{q_{ph}} \quad \text{(A5)}$$

Here, q_{ph} is the specific light absorption rate ($mol_{ph} mol_x^{-1} s^{-1}$), which is defined as follows:

$$q_{ph} = a_x \cdot I_{ph} \quad \text{(A6)}$$

The light intensity at which μ is equal to zero (i.e., where photosynthetic sugar production is compensated by maintenance-associated sugar consumption) is referred to as the photosynthetic compensation point ($I_{ph,c}$). By numerical integration of $Y_{x/ph}$ from $I_{ph}=I_{ph,c}$ to $I_{ph}=1500$ μmol photons $m^{-2}\ s^{-1}$, the maximal biomass productivity per illuminated surface area (r_x) is obtained in $g\ m^{-2}\ h^{-1}$ (Eq. A7).

$$r_x = 3600 \cdot 10^{-6} \cdot \sum_{I_{ph,c}}^{I_{ph,in}} Y_{x/ph} \cdot \Delta I_{ph} \quad \text{(A7)}$$

Appendix 2. Light intensity distribution over reactor surface

Table 3 Incident light intensity (μmol photons $m^{-2}\ s^{-1}$) distribution over the reactor surface. The light was measured at 28 points evenly distributed over the light exposed surface of the front glass panel of the culture chamber. For this measurement, the average light intensity was 1501 μmol photons $m^{-2}\ s^{-1}$

1235	1429	1352	1286
1464	1665	1656	1409
1683	1837	1737	1739
1586	1882	1953	1571
1601	1738	1670	1438
1383	1568	1591	1177
1001	1135	1144	1099

Appendix 3. Calculation of the extrapolated biomass concentration C_x' using the attenuation coefficient K_x

For each mutant, the spectrally averaged attenuation coefficient (K_x, $m^2\ g^{-1}$) of the experiment with the darkest light regime (i.e., lowest $I_{ph,out}$) was calculated. This attenuation coefficient K_x was determined by measuring the light transmittance in the photobioreactor by measuring the $I_{ph,in}$ and $I_{ph,out}$ using a PAR light meter. This attenuation coefficient K_x, therefore, also includes the effect of light scattering as it occurs in the photobioreactor. The coefficient K_x is fundamentally different from the spectrally averaged optical cross section (a_x) which only reflects true light absorption. K_x was calculated using the equations indicated below in which d (m) is the light path of the photobioreactor.First, K_x is calculated using Eq. C1 which was obtained by rearranging Eq. C2, Lambert-Beer's law equation.

$$K_x = \frac{\ln \frac{I_{ph,out}}{I_{ph,in}}}{-C_x \cdot d} \tag{C1}$$

$$I_{ph,out} = I_{ph,in} \cdot e^{-C_x \times K_x \times d} \tag{C2}$$

With K_x known, C_x' at $I_{ph,out}$=10 μmol photons $m^{-2}\ s^{-1}$ can be estimated using Eq. C3, which was also obtained by rearranging Eq. C2.

$$C_x' = \frac{\ln \frac{I_{ph,out}}{I_{ph,in}}}{-K_x \cdot d} \tag{C3}$$

References

Alboresi A, Ballottari M, Hienerwadel R, Giacometti GM, Morosinotto T (2009) Antenna complexes protect photosystem I from photoinhibition. BMC Plant Biol 9:71

Blanken W, Cuaresma M, Wijffels RH, Janssen M (2013) Cultivation of microalgae on artificial light comes at a cost. Algal Res 2:333–340

Bonente G, Formighieri C, Mantelli M, Catalanotti C, Giuliano G, Morosinotto T, Bassi R (2011) Mutagenesis and phenotypic selection as a strategy toward domestication of *Chlamydomonas reinhardtii* strains for improved performance in photobioreactors. Photosynth Res 108:107–120

Cuaresma M, Janssen M, Vílchez C, Wijffels RH (2011) Horizontal or vertical photobioreactors? How to improve microalgae photosynthetic efficiency. Bioresource Technol 102:5129–5137

De Vitry C, Wollman F-A (1988) Changes in phosphorylation of thylakoid membrane proteins in light-harvesting complex mutants from *Chlamydomonas reinhardtii*. BBA-Bioenerg 933: 444–449

Deblois CP, Marchand A, Juneau P (2013) Comparison of photoacclimation in twelve freshwater photoautotrophs (Chlorophyte, Bacillaryophyte, Cryptophyte and Cyanophyte) isolated from a natural community. PLoS One 8:e57139

Dubinsky Z, Falkowski PG, Wyman K (1986) Light harvesting and utilization by phytoplankton. Plant Cell Physiol 27:1335–1349

Duboc P, Marison I, Von Stockar U (1999) Quantitative calorimetry and biochemical engineering. Handb Therm Anal Calorim 4:267–365

Dye DJ (2010) Spatial light dilution as a technique for conversion of solar energy to algal biomass. Dissertation, Utah State University

Formighieri C, Franck F, Bassi R (2012) Regulation of the pigment optical density of an algal cell: filling the gap between photosynthetic productivity in the laboratory and in mass culture. J Biotechnol 162:115–123

Grewe S, Ballottari M, Alcocer M, D'Andrea C, Blifernez-Klassen O, Hankamer B, Mussgnug JH, Bassi R, Kruse O (2014) Light-harvesting complex protein LHCBM9 is critical for photosystem II activity and hydrogen production in *Chlamydomonas reinhardtii*. Plant Cell 26:1598–1611

Grossman AR, Bhaya D, Apt KE, Kehoe DM (1995) Light-harvesting complexes in oxygenic photosynthesis: diversity, control, and evolution. Annu Rev Genet 29:231–288

Huesemann MH, Hausmann TS, Bartha R, Aksoy M, Weissman JC, Benemann JR (2009) Biomass productivities in wild type and pigment mutant of Cyclotella sp. (Diatom). Appl Biochem Biotechnol 157:507–526

Hutner S, Provasoli L, Schatz A, Haskins C (1950) Some approaches to the study of the role of metals in the metabolism of microorganisms. Proc Am Philos Soc 94:152–170

Janssen M, Janssen M, de Winter M, Tramper J, Mur LR, Snel J, Wijffels RH (2000) Efficiency of light utilization of *Chlamydomonas reinhardtii* under medium-duration light/dark cycles. J Biotechnol 78:123–137

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21:540–547

Kirst H, García-Cerdán JG, Zurbriggen A, Melis A (2012) Assembly of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* requires expression of the TLA2-CpFTSY gene. Plant Physiol 158:930–945

Kliphuis AM, Klok AJ, Martens DE, Lamers PP, Janssen M, Wijffels RH (2012) Metabolic modeling of *Chlamydomonas reinhardtii*: energy requirements for photoautotrophic growth and maintenance. J Appl Phycol 24:253–266

Kwon J-H, Bernát G, Wagner H, Rögner M, Rexroth S (2013) Reduced light-harvesting antenna: consequences on cyanobacterial metabolism and photosynthetic productivity. Algal Res 2:188–195

Liberton M, Collins AM, Page LE, O'Dell WB, O'Neill H, Urban VS, Timlin JA, Pakrasi HB (2013) Probing the consequences of antenna modification in cyanobacteria. Photosynth Res 118:17–24

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

Melis A (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci 177:272–280

Mussgnug JH, Thomas-Hall S, Rupprecht J, Foo A, Klassen V, McDowall A, Schenk PM, Kruse O, Hankamer B (2007) Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion. Plant Biotechnol J 5:802–814

Nakajima Y, Ueda R (1998) Improvement of photosynthesis in dense microalgal suspension by reduction of light harvesting pigments. J Appl Phycol 9:503–510

Nakajima Y, Ueda R (2000) The effect of reducing light-harvesting pigment on marine microalgal productivity. J Appl Phycol 12: 285–290

Nakajima Y, Tsuzuki M, Ueda R (2001) Improved productivity by reduction of the content of light-harvesting pigment in *Chlamydomonas perigranulata*. J Appl Phycol 13:95–101

Norsker N-H, Barbosa MJ, Vermuë MH, Wijffels RH (2011) Microalgal production—a close look at the economics. Biotechnol Adv 29:24–27

Oey M, Ross IL, Stephens E, Steinbeck J, Wolf J, Radzun KA, Kügler J, Ringsmuth AK, Kruse O, Hankamer B (2013) RNAi knock-down of LHCBM1, 2 and 3 increases photosynthetic H_2 production efficiency of the green alga *Chlamydomonas reinhardtii*. PLoS One 8: e61375

Olive J, Wollman F-A, Bennoun P, Recouvreur M (1981) Ultrastructure of thylakoid membranes in *C. reinhardtii*: evidence for variations in the partition coefficient of the light-harvesting complex-containing particles upon membrane fracture. Arch Biochem Biophys 208: 456–467

Ort DR, Zhu X, Melis A (2011) Optimizing antenna size to maximize photosynthetic efficiency. Plant Physiol 155:79–85

Peers G, Truong TB, Ostendorf E, Busch A, Elrad D, Grossman AR, Hippler M, Niyogi KK (2009) An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. Nature 462: 518–521

Perrine Z, Negi S, Sayre RT (2012) Optimization of photosynthetic light energy utilization by microalgae. Algal Res 1:134–142

Pirt S (1965) The maintenance energy of bacteria in growing cultures. Proc R Soc Lond B163:224–231

Polle JE, Kanakagiri S-D, Melis A (2003) tla1, a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size. Planta 217:49–59

Takache H, Christophe G, Cornet JF, Pruvost J (2010) Experimental and theoretical assessment of maximum productivities for the microalgae *Chlamydomonas reinhardtii* in two different geometries of photobioreactors. Biotechnol Progr 26:431–440

Tanaka R, Tanaka A (2000) Chlorophyll b is not just an accessory pigment but a regulator of the photosynthetic antenna. Porphyrins 9:240–245

Tokutsu R, Minagawa J (2013) Energy-dissipative supercomplex of photosystem II associated with LHCSR3 in *Chlamydomonas reinhardtii*. Proc Natl Acad Sci U S A 110:10016–10021

Tokutsu R, Iwai M, Minagawa J (2009) CP29, a monomeric light-harvesting complex II protein, is essential for state transitions in *Chlamydomonas reinhardtii*. J Biol Chem 284:7777–7782

Tredici MR, Zittelli GC (1998) Efficiency of sunlight utilization: tubular versus flat photobioreactors. Biotechnol Bioeng 57:187–197

Vejrazka C, Janssen M, Benvenuti G, Streefland M, Wijffels RH (2013) Photosynthetic efficiency and oxygen evolution of *Chlamydomonas reinhardtii* under continuous and flashing light. Appl Microbiol Biotechnol 97:1523–1532

Zemke PE, Sommerfeld MR, Hu Q (2013) Assessment of key biological and engineering design parameters for production of *Chlorella zofingiensis* (Chlorophyceae) in outdoor photobioreactors. Appl Microbiol Biotechnol 97:5645–5655