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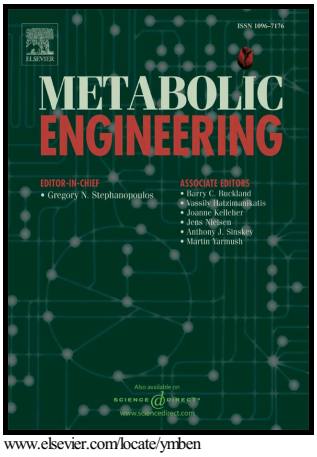

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A Mathematical model to guide Genetic Engineering of Photosynthetic Metabolism

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Abstract

The optimization of algae biomass productivity in industrial cultivation systems requires genetic improvement of wild type strains isolated from nature. One of the main factors affecting algae productivity is their efficiency in converting light into chemical energy and this has been a major target of recent genetic efforts. However, photosynthetic productivity in algae cultures depends on many environmental parameters, making the identification of advantageous genotypes complex and the achievement of concrete improvements slow.

In this work, we developed a mathematical model to describe the key factors influencing algae photosynthetic productivity in a photobioreactor, using experimental measurements for the WT strain of *Nannochloropsis gaditana*. The model was then exploited to predict the effect of potential genetic modifications on algae performances in an industrial context, showing the ability to predict the productivity of mutants with specific photosynthetic phenotypes. These results show that a quantitative model can be exploited to identify the genetic modifications with the highest impact on productivity taking into full account the complex influence of environmental conditions, efficiently guiding engineering efforts.

ABBREVIATIONS

ASII, antenna size of photosystem II; Chl, chlorophyll content of an algae cell; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; N, inverse of the Emerson and Arnold number; NPQ, non-photochemical quenching; P, oxygen evolution rate; PBR, photobioreactor; PI, photosynthesis-irradiance; PSII, photosystem II; PSU, photosynthetic unit; RCII, reaction centers of photosystem II; ROS, reactive oxygen species; TAGs, triacylglycerols; WT, wild type; σ, chlorophyll specific total cross section of the photosystems; τ, turnover rate of the PSII

KEYWORDS

Photosynthesis, Modeling, biomass productivity, photobioreactor, *Nannochloropsis*

1. INTRODUCTION

Global demand for products derived from biomass such as food, feed or fuels is continuously expanding and not satisfactorily fulfilled. New technologies to improve the current biomass production are thus strongly needed to meet this increasing demand while also improving sustainability. One major strategy to be pursed is the development of technologies to exploit new biomass sources to complement plant crops. Microalgae are highly promising in this context since they are diverse, unicellular, eukaryotic photosynthetic organisms that use sunlight to produce biomass and oxygen from carbon dioxide (CO_2),

water, and nutrients. They can be cultivated in marginal or saline water on unproductive land and thus do not compete with agriculture for arable soils and freshwater, making them a promising resource for a highly sustainable production of biomass (Gangl et al., 2015; Leu and Boussiba, 2014). Microalgae advantages are largely recognised and several promising applications have been suggested (e.g. bio-plastics, feed and biofuels) and developed, but the potential of these organisms still remains largely untapped. Significant improvement in strains performances and cultivation technologies is in fact strongly required to reduce production cost of algal biomass, which is currently limiting their exploitation to the production of only high-value bio-products. Efforts in this direction are thus seminal to expand the possible applications of algae biotechnology to commodities with a lower market value.

Algae large scale cultures are commonly limited by available irradiation and a main target of optimization efforts aims to improve the efficiency in converting light energy into reduced carbon. Wild type (WT) algae adapted during evolution to a natural environment with largely different conditions than those found during industrial cultivation in a photobioreactor (PBR) and thus genetic modifications have a seminal potential to improve productivity. As example, in nature algae often deal with limiting light conditions and accumulate a large number of chlorophyll binding proteins to compete with other organisms for energy capture. This ability is detrimental in intensive cultivation systems where algae reach high cellular densities and external layers absorb most of the available energy leaving the rest of the culture in strong light limitation. On the other hand, algae also evolved mechanisms (e.g. non-photochemical quenching, NPQ) to deal with excess irradiation to avoid over-excitation and the generation of reactive oxygen species (ROS) that compromise photosynthesis functionality. These mechanisms are especially activated by external cells more exposed to illumination, which thus activate NPQ with a reduction of their light use efficiency (Simionato et al., 2013) and dissipation of up to 80% of absorbed energy as heat (Peers et al., 2009).

Algae thus need to be domesticated to optimize their productivity upon cultivation in industrial conditions. Several modifications of the photosynthetic apparatus were proposed in recent years to improve light homogeneity inside the culture and consequently productivity (Formighieri et al., 2012; Wobbe et al., 2015). Among them, one often suggested strategy is the generation of strains with a reduced chlorophyll

(Chl) content, leading to more transparent mass cultures where light is more homogenously distributed and overall productivity higher. . Another proposed strategy is to reduce the number of antenna complexes, the chlorophyll (Chl) binding proteins bound to the two photosystems, that are responsible for most of the light harvesting (Dall'Osto et al., 2015; Polle et al., 2002). Out of the ~300 chlorophyll molecules bound to both photosystems, in fact, only about one third are associated to the core complexes and thus involved in fundamental reactions of the photosynthesis, while the others are in principle dispensable (Melis, 1991). In a mass culture context a reduction in antenna content should reduce cells light harvesting ability but also prevent over-excitation in excess light conditions, increasing the amount of light energy exploitable at high efficiency (Mitra et al., 2012; Simionato et al., 2013; Wobbe and Remacle, 2014). A third proposed strategy is the reduction of NPQ which, by channelling more energy into photochemistry rather than wasting it through heat dissipation, could lead to increase the efficiency of light energy conversion in biomass (Berteotti et al., 2016; Erickson et al., 2015).

Genetic modifications of algae photosynthetic properties have been shown to be beneficial for productivity in lab scale conditions by several groups, including ours (Berteotti et al., 2016; Kirst et al., 2012; Perin et al., 2015). Attempts to validate the improved productivity in larger scale cultivation were successful in some cases but not others (Cazzaniga et al., 2014; de Mooij et al., 2014). One major explanation for the different results is that environmental conditions experienced in large scale cultivation are significantly different from lab scale and have a major impact on performances of genetically improved strains (Perin et al., submitted). Algae cultivated in a PBR / pond are in fact exposed to a complex environment, where many different parameters influence productivity, impairing the possibility of extrapolating the impact of a genetic modification from simple lab scale evaluations.

On the other hand, productivity evaluations on a large scale are very time consuming and expensive, thus discouraging a trial and error approach and supporting the need of a more rational approach. One strategy to tackle this issue is to develop mathematical models capable of describing quantitatively the influence of major cultivation parameters on algae performances. This objective is particularly intricate since metabolism of light-energy conversion spans numerous time-scales ranging from milliseconds to days. We

developed a model capable of reliably describing photosynthetic light conversion, accounting for processes like photoproduction, photoinhibition and photo-regulation (Bernardi et al., 2016; Nikolaou et al., 2015). In the present work, this model was further modified to account for the light distribution in a PBR, achieving a reliable description of algae photosynthetic productivity in an industrially relevant environment. This model was exploited to predict the effect of genetic modifications of the photosynthetic apparatus and to assess their impact on biomass productivity during large scale cultivation. These predictions were verified with experimental data from three independent mutant strains, demonstrating for the first time that modelling approaches can be exploited for assessing the effectiveness of a genetic modification, making the identification of the ones providing the largest advantages faster and more effective.

2. MATERIALS AND METHODS

Nannochloropsis gaditana was selected for this work because it is widely recognized as model organism for algae industrial applications, because of its ability to accumulate triacylglycerols (TAGs) (Bondioli et al., 2012; Rodolfi et al., 2009; Simionato et al., 2013).

2.1 Culture conditions and growth determination

2.1.1 Strains. Nannochloropsis gaditana, strain 849/5, from the Culture Collection of Algae and Protozoa (CCAP) was used as the WT strain. Mutants with altered photosynthetic apparatus were isolated using random mutagenesis approaches, as previously described in (Perin et al., 2015). Three of most promising strains (E2, I48 and I29) were employed in this work.

2.1.2 Cultivation conditions. All strains were maintained in F/2 solid media, with sea salts (32 g/L, Sigma Aldrich), 40 mM Tris-HCl (pH 8), Guillard's (F/2) marine water enrichment solution (Sigma Aldrich), 1 % agar (Duchefa Biochemie). Cells were pre-cultured in sterile F/2 liquid media in Erlenmeyer flasks with 100 µmoles photons m^{-2} s^{-1} illumination and 100 rpm agitation at 22 ± 1 °C in a growth chamber. Growth tests in PBR simulating industrial cultivation (later on we will refer to these growth conditions as "industrial conditions" for brevity), were performed with semi-continuous cultures in 5 cm diameter Drechsel bottles

with a 250-ml working volume. Bottles were illuminated from one side (illumination rate was determined using the LI-250A photometer (Heinz-Walz, Effeltrich, Germany)) and bubbled using air enriched with 5 % CO_2 (v/v), at a total flow rate of 1 L h^{-1}, as detailed in (Sforza et al., 2012a). In this case, F/2 growth media was enriched with added nitrogen, phosphate and iron sources (0.75 g/L $NaNO_3$, 0.05 g/L NaH_2PO_4 and 0.0063 g/L $FeCl_3 \bullet 6\ H_2O$ final concentrations). Constant illumination (24 h/24 h) at 400 µmoles photons m^{-2} s^{-1} was provided by a white LED light source SL 3500 (Photons Systems Instruments, PSI, Brno, Czech Republic). Irradiance conditions were chosen in order to expose cells populating the external layers of the PBR to saturating light conditions, while leaving the inner layers in light limitation, to highlight the effects of inhomogeneous distribution. The pH of the semi-continuous cultures was set to 8.00 and fresh media was added every other day to restore the starting cell concentration to values between 150 and 250 x 10^6 cells ml^{-1} (corresponding to biomass concentration values between 1.0 and 1.8 g L^{-1}). Cells growth monitored after 1, 2 and 3 days was indistinguishable, demonstrating that nutrients and CO_2 were not limiting. All data presented were collected after 2 days of cultivation to ensure that cells were actively growing in stable environmental conditions.

2.1.3 Analytical procedures and energy balance. Algal growth was monitored using a cell counter (Cellometer Auto X4, Nexcelom Bioscience) and evaluating biomass concentration. The specific growth rate was calculated by the slope of logarithmic phase for the biomass concentration (dry weight) measured gravimetrically as previously reported in (Perin et al., 2015). Biomass productivity was calculated as ($[C_f]$ - $[C_i]$) / (t_f - t_i), where C is the final (f) (before dilution) or initial (i) (after dilution) biomass concentration of the culture and t is the growth time interval, expressed as number of days. The specific light supply rate per unit mass of cell, r_{Ex} (mmoles photons g^{-1} d^{-1}), in the growth conditions of this work was calculated according to (Sforza et al., 2015) as:

$$r_{Ex} = \frac{PFD_{abs} \cdot A_{PBR}}{C_x \cdot V_{PBR}} \qquad (1)$$

where PFD_{abs} is the PAR photon flux density absorbed by the culture (µmoles photons m^{-2} s^{-1}), A_{PBR} is the irradiated surface of the reactor (m^2), C_x is the microalgae concentration inside the PBR (g/L) and V_{PBR} is the volume of the culture.

2.2 Pigments extraction

Pigments were extracted from cells grown in semi-continuous conditions, during active growth. Chlorophyll a was extracted from intact cells, using a 1:1 biomass to solvent ratio of 100 % N,N-dimethylformamide (Sigma Aldrich) (Moran and Porath, 1980), at 4 °C in the dark, for at least 24 h. Absorption spectra were registered between 350 and 750 nm using a Cary 100 spectrophotometer (Agilent Technologies) to spectrophotometrically determine pigment concentrations, using specific extinction coefficients (Porra et al., 1989; Wellburn, 1994). Absorption values at 664 were used to calculate the concentrations of Chlorophyll a.

2.3 Photosynthetic performances.

Photosynthesis monitoring was performed by measuring *in vivo* Chl fluorescence using a PAM 100 fluorimeter (Heinz-Walz, Effeltrich, Germany). Samples were treated with increasing light intensity up to 2000 µmoles photons m^{-2} s^{-1} and then light was switched off to evaluate NPQ relaxation kinetic. NPQ was calculated as in (Demmig-Adams et al., 1989; Maxwell and Johnson, 2000).

2.3.1 PSII functional antenna size evaluation. PSII antenna sizes were determined for semi-continuous cultures in active growth conditions, using a JTS-10 spectrophotometer (Biologic, France). Samples (200 x 10^6 cells/ml final concentration) were dark-adapted for 20 minutes and incubated with 80 µM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) for 10 minutes. Fluorescence induction kinetics were then monitored upon excitation with 320 µmoles photons m^{-2} s^{-1} of actinic light at 630 nm. The $t_{2/3}$ values obtained from the fluorescence induction curves were used to calculate the size of the PSII functional antenna (Perin et al., 2015).

2.4 Mathematical modelling.

Simulations of the growth model were conducted in the modelling environment gPROMS (Process System Enterprise, gPROMS v 4.1, www.psenterprise.com/gproms, 1997-2016). Model parameters for the wild type strain were obtained in (Bernardi et al., 2016) using the maximum likelihood optimisation criterion.

2.5 Statistical analysis.

We applied descriptive statistical analysis with mean and standard deviation for all the experimental data reported in this work. Statistical significance was evaluated by one-way analysis of variance (One-way ANOVA), using the OriginPro 8 software (http://www.originlab.com/). Samples size was > 40 for all the measurements performed in this work.

3. THEORY AND CALCULATION

3.1 Model for microalgae photosynthetic productivity.

The photosynthetic productivity of *Nannochloropsis gaditana* WT strain, as function of the incident irradiation, was described according to a previously developed model (Bernardi et al., 2016; Nikolaou et al., 2015). A detailed description of the previous model is already available at the indicated references. However, for the sake of clarity, we included below a summary of the overall set of equations of the previous model as reference; all the model parameters and corresponding units are shown in supplementary table S1.

$$F = S_F \sigma \Phi_f \quad (2)$$

$$\Phi_f = \frac{1}{\frac{A}{\Phi_f^A} + \frac{B}{\Phi_f^B} + \frac{C}{\Phi_f^C}} \quad (3)$$

$$\dot{A} = -I\sigma_{PSII}A + \frac{1}{\tau}B \quad (4)$$

$$\dot{B} = I\sigma_{PSII}A - \frac{1}{\tau}B + k_r C - k_d \sigma_{PSII} IB \quad (5)$$

$$\dot{C} = k_d \sigma_{PSII} IB - k_r C \quad (6)$$

$$\sigma_{PS2} = \frac{\sigma}{\upsilon\, N}\Phi_P^A \quad (7)$$

$$\Phi_P^A = \eta_P \Phi_f^A \quad (8)$$

$$\Phi_f^A = \frac{1}{1+\eta_P+\eta_D+\eta_{qE}} \quad (9)$$

$$\Phi_f^B = \frac{1}{1+\eta_D+\eta_{qE}} \quad (10)$$

$$\Phi_f^C = \frac{1}{1+\eta_I+\eta_D+\eta_{qE}} \quad (11)$$

$$\dot{\alpha}_F = \xi_F\,(\alpha_{SS} - \alpha_F) \quad (12)$$

$$\dot{\alpha}_S = \xi_S\,(\alpha_{SS} - \alpha_S) \quad (13)$$

$$\alpha_{SS} = \frac{I^n}{I_{qE}^n + I^n} \quad (14)$$

$$\eta_{qE} = \alpha_F\left(\bar{\eta}_{qE}^F + \alpha_S\,\bar{\eta}_{qE}^C\right) + \alpha_S\,\bar{\eta}_{qE}^S \quad (15)$$

$$P = \theta\,\sigma\Phi_P I \quad (16)$$

Equation 2 defines the fluorescence flux, *F* (V), as a function of the total cross-section, σ ($m^2\ g^{-1}_{chl}$), the fluorescence quantum yield, ϕ_f (−), and a parameter depending on the characteristics of the pulse amplitude modulation (PAM) fluorometer and the chlorophyll content of the sample, S_F ($Vg_{chl}\ m^{-2}$). The fluorescence quantum yield given in equation 3 is the harmonic mean of the fluorescence quantum yields of open (A), closed (B) and damaged (C) reaction centers of photosystem II (RCII). The dynamics of A, B and C are described according to the Han model (Han et al., 2000) in equations 4-6, according to the parameters: the effective cross-section of the PSII, σ_{PSII} ($m^2\ \mu E^{-1}$), the turnover rate, τ (s^{-1}), the damage rate constant, k_d (−), and the repair rate constant k_r (s^{-1}). Equation 7 relates the effective cross-section, σ_{PSII} of the Han model with σ, linking the state of the RCII with the fluorescence flux. It is worth noting that while σ_{PSII} is a constant in the Han model, here it becomes a function of the photoregulation activity (equation 15), via the quantum yield of photosynthesis of an open RCII (equations 8 and 9). The parameter *N* represents the chlorophyll specific number of photosynthetic units ($\mu moles_{O2}\ g^{-1}chl$), and *υ* is a stoichiometric factor reflecting the minimum theoretical value of 4 electrons for each dissociated water molecule, according to the water dissociation reaction ($2H_2O + 4e^- \rightarrow O_2 + 4H^+$).

Equation 10 defines the quantum yield of photosynthesis of an open RCII. Equations 11-13 define the quantum yield of fluorescence of the open, closed and inhibited photosynthetic units (PSUs), with the parameters η_P, η_D and η_I representing the rates of photoproduction, basal thermal decay in dark-adapted state and qI-quenching, respectively. Parameter η_{qE} represents the combined quenching effect of light harvesting complex (LHC) proteins involved in photoprotection (LHCX in *Nannochloropsis* species (Alboresi et al., 2016; Vieler et al., 2012)) and zeaxanthin, and it is calculated from equation 15, where the parameters $\bar{\eta}_{qE}^{F}$ and $\bar{\eta}_{qE}^{S}$ represent the rates of the fast (LHCX-related) and slow (zeaxanthin-related) components of the non-photochemical-quenching (NPQ) process, both relative to the rate of fluorescence; the parameter $\bar{\eta}_{qE}^{C}$represents the enhancing effect of zeaxanthin in the quenching capability of the LHCX proteins. The activities of the fast and slow NPQ components are described by the conceptual variables α_F and α_S, both modelled as first-order processes. Moreover, the reference α_{SS} is the same in equations 12 and 13 since LHCX- and zeaxanthin-related quenching are both triggered by low lumenal pH (Pinnola et al., 2013), yet with different time constants ξ_F and ξ_S. This reference is modelled by a sigmoid (Hill) function in equation 14, in agreement with experimental measurements of the NPQ index as function of *I*, according to (Kramer et al., 2004), with I_{qE} and *n* representing the irradiance level at which half of the maximal NPQ activity is triggered (α_{SS} = 0.5) and the sharpness of the switch-like transition, respectively.

Finally, the maximum and minimum fluorescence fluxes, F'_m and F'_0, can be calculated from equations 2 and 3, by varying A and B. Specifically F'_m is obtained by imposing A = 0 and B = 1 − C, whereas F'_0 by imposing A = 1 − C and B = 0. Moreover, the distinction between dark- and light-adapted fluxes depends on the value of the variables α_F and α_S, assuming $\alpha_F = \alpha_S = 0$ for the dark-adapted state.

Equation 16 describes the photosynthetic productivity, *P* [$g_{O2}/g_{chl}h$], as a function of the model parameter σ and the light intensity trough the variable Φ_P defined as $\frac{1}{\nu}\frac{F'_m - F'}{F'_m}$. In order to express *P* in grams of oxygen produced per gram of chlorophyll per hour a conversion factor θ, equal to 0.115, is applied.

3.2 Conversion of photosynthetic productivity in growth rate.

The model described in 3.1 expresses microalgae net photosynthetic productivity as oxygen evolution rate, P [$g_{O2}/g_{chl}h$]. In order to predict microalgae biomass growth, the oxygen productivity should be converted into the growth rate constant, μ [d^{-1}], also accounting for the energy spent for maintenance. This was done according to the product of four terms:

- A conversion factor of 0.76 grams of biomass ($g_{biomass}$) per gram of O_2 (g_{O2}) produced (Béchet et al., 2015);
- The [Chl]/ cell, which is $1.2 \cdot 10^{-13}$ g for the microalgae cultivated in the intensive growth conditions of this work;
- The inverse of the cell weight, which corresponds to $1.5 \cdot 10^{11}$ g^{-1};
- A factor of 24 to have the growth rate constant in d^{-1}.

The resulting equation to calculate the growth rate constant is thus the following:

$$\mu = 0.33\ (P - M) \qquad (17)$$

Where the respiration factor *M* has been estimated from the negative slope of the oxygen evolution rate during the initial dark phase of each experiment, and is equal to 0.2 [$g_{O2}/g_{chl}h$].

3.3 Modelling of light attenuation in algal culture and model validation

To reliably describe the light attenuation profile inside the PBR the Lambert-Beer law is the simplest model available. This model assumes that the light intensity, *I* (*z*), decreases exponentially with the culture depth, *z*, according to equation 18:

$$I(z) = I(0)\exp(-K_{LB}z) \qquad (18)$$

where K_{LB} is the light attenuation coefficient, assumed to be linearly related with the biomass concentration of the sample. Since this is not the case in highly concentrated samples (> 1 g L^{-1}), we moved on the model by Yun et al. (Yun and Park, 2001) that overcomes this limitation and includes both absorption and scattering phenomena. This empirical model assumes a hyperbolic form to describe the variation of K_{LB} with the biomass:

$$K_{LB}(x_{biom}) = K_{LB}^{max} \frac{x_{biom}}{x_{biom} + k_B} \quad (19)$$

where K_{LB}^{max} is the maximum absorption coefficient and k_B is a constant. According to this model, at low biomass concentrations, the attenuation change is linear with (x_{biom}), while at high biomass concentration it shows an asymptotic tendency towards a maximum.

Model calibration was performed by assessing the light absorbed by microalgae samples at different cell concentrations, using a 1-cm-path-length cuvette, irradiated from a fixed distance with increasing light intensities (Supplementary Table S2) with a halogen lamp (KL1500, Schott, Germany). For each sample, $K_{LB}(x_{biom})$ was calculated as:

$$K_{LB}(x_{biom}) = \frac{1}{h} \ln\left(\frac{I^w(h)}{I(h, x_{biom})}\right) \quad (20)$$

where h is the path length, $I^w(h)$ is the light measured using a cuvette filled with water (blank) and $\mathrm{I}\,(h, x_{biom})$ is the light measured when the cuvette is filled with the microalgae sample.

3.3.1 Light attenuation profile for photosynthetic mutants. The effects of hypothetical alterations of photosynthetic apparatus in mutants on light attenuation profile inside the PBR were described by modifying the light attenuation coefficient as:

$$K_{LB}^{mut} = K_{LB}^{WT} \frac{chl_{mut}\sigma_{mut}}{chl_{WT}\sigma_{WT}} \quad (21)$$

where K_{LB}^{mut} and K_{LB}^{WT} are the light attenuation coefficients for the mutants and the wild type, respectively, chl_{mut} and chl_{WT} represent the chlorophyll contents of mutants and the wild type, respectively and σ_{mut} and σ_{WT} are the chlorophyll specific total cross sections of the photosystems in mutants and wild type, respectively.

3.4 Modelling microalgae growth in PBR

In order to predict the growth of *Nannochloropsis gaditana* WT strain in PBR, we combined the model developed in (Bernardi et al., 2016) and here implemented to convert photosynthetic productivity in specific growth rate as function of the incident irradiation (see 3.2 for details), with the model describing the light attenuation profile in PBR developed in this work and presented in 3.3.

To account for the inhomogeneous light distribution in the mass culture:

- The photobioreactor was divided in ten discrete sub-regions, based on the distance from the half-circumference illuminated by the light source (see Supplementary Figure S1 for the scheme). In each region a constant light intensity equal to the value predicted from the light attenuation model at the median point of the corresponding area was assumed. Therefore, as the biomass concentration increases during growth, light attenuation is expected to increase as a function of time.
- The model predictions of the growth rate constant in each region of the PBR were used to calculate the biomass concentration variations, assuming that cultures remain in an exponential growth phase;
- The biomass concentration inside the photobioreactor was obtained as the volumetric mean of the biomass concentrations in the different regions;
- The average growth rate constant was calculated according to the following equation:

  $$\mu(T) = \frac{1}{T}\ln\left(\frac{x_{biom}(T)}{x_{biom}(0)}\right) \qquad (22)$$

  where *T* is the growth time interval, expressed in days (d).

3.5 Model implementation to predict the growth of photosynthetic mutants in PBR

In order to predict the effect on growth in PBR of mutants showing alterations on photosynthesis, we modified a subset of model parameters values earlier estimated to describe WT photosynthetic productivity (see 3.1, (Bernardi et al., 2016)). For instance, to simulate *in silico* a mutant with a reduced

NPQ activation, it is sufficient to reduce the values of the parameters $\bar{\eta}_{qE}^{F}$, $\bar{\eta}_{qE}^{S}$ and $\bar{\eta}_{qE}^{C}$, which describe the different components of photo-regulation of microalgae cells, according to equation 15 of 3.1.

Modifications of photosystem II (PSII) functional antenna size (ASII) and cell chlorophyll content are instead expected to lead to a variation of the following model parameters: σ, τ and N, representing the chlorophyll specific total cross section of the photosystems, the turnover rate of the PSII and the inverse of the Emerson and Arnold number (a measurement of the chlorophyll molecules associated with each reaction center of PSII), respectively. In particular:

- The antenna size in the model is represented by the ratio between σ and N, therefore to represent a reduction of antenna size this ratio has to be modified accordingly;
- The reduction of *Chl* might lead to an increase in σ due to a lower self-shading effect between chlorophyll molecules (Falkowski and Raven, 2013);
- The parameter τ can decrease if the chlorophyll reduction leads to a reduction of the number of reaction centers (Falkowski and Raven, 2013).

According to these considerations, we set the parameter σ varying linearly as function of the chlorophyll content of the cells, according to the following equation:

$$\sigma = \sigma_{WT} + \sigma_{WT} \frac{chl_{WT} - chl}{chl_{WT}} \quad (23)$$

with and the relative σ variation being equal to the opposite of the relative Chl variation. It should be noted that this equation would lose accuracy in case of strong Chl increases but the possibility of using a more complex description was discarded to avoid introducing additional parameters since strains with increased pigment accumulation are not considered here.

This assumption was validated using *Nannochloropsis gaditana* photosynthetic mutants with altered Chl content (strains E2 and I48 of this work) observing that the ratio between σ_{mut} and σ_{WT} is always greater

than one in high density cultures (cells concentration > 150 x 10^6 cells/ml, corresponding to > 1 g L^{-1}), indicating that σ indeed increases as chlorophyll content decreases (see supplementary figure S2).

Another parameter that can vary according to the variations of Chl and ASII is the maximum turnover rate, τ. If we consider that Chl $\propto$ ASII$\cdot X_N$, where X_N is the number of photosynthetic units, a reduction in the values of Chl content greater than the reduction of ASII is consistent with a lower number of reaction centres, which is also expected to lead to a decrease of the maximum turnover rate (Falkowski, 1993). Therefore, we set the maximum turnover rate, τ, varying as function of the both the chlorophyll content and the ASII of the cell, according to the following mathematical relation:

$$\tau = \tau_{WT}\left(0.1 + (1-0.1) * \frac{chl}{chl_{WT}}\frac{ASII_{WT}}{ASII}\right) \qquad (24)$$

with the variation of τ being directly proportional to the variation of the ratio Chl*/ASII.* To assure that the parameter τ is always greater than zero an intercept of 0.1 has been imposed. The proposed formulation assures that if Chl and *ASII* are equal to the WT values, then $\tau = \tau_{WT}$ and that τ can be reduced maximum by one order of magnitude, which is consistent with the data reported in (Falkowski and Raven, 2013).

4. RESULTS

4.1 Quantitative description of light attenuation in an algae culture in PBR.

Because of shading and scattering effects in an algae culture within a PBR, light intensity reaching each cell is highly different and different layers contribute differently to biomass productivity. Several models have been proposed in literature to describe absorption and scattering phenomena (Cornet et al., 1995, 1992; Yokota et al., 1991) and can be exploited to describe quantitatively the light attenuation profile in a PBR. We selected the one by Yun et al. (see 3.3 for details on the equations) that was demonstrated to describe accurately the light profile in an algae mass culture (Yun and Park, 2001). The model by Yun et al. was calibrated using a series of experimental measurements of light absorbed by a microalgae sample in dependence from cell concentration and the incident light intensity (see 3.3 and Supplementary Table S2

for data). As shown in Figure 1, following this approach it was possible to obtain a satisfactory correspondence between experimental data and model estimations of the light attenuation coefficient (K_{LB}) inside a culture in PBR for *N. gaditana* wild type cells.

4.2 Modelling growth of Nannochloropsis gaditana wild type strain in a PBR.

In order to describe quantitatively algae biomass productivity in intensive growth conditions, the mathematical description of the light attenuation profile inside a PBR (4.1) was combined with the model describing the dependence of algae photosynthetic productivity from incident irradiation (Bernardi et al., 2016) (see 3.1, 3.2 and 3.4 for details), including the photosynthetic performances of algal cells as a function of the available irradiation and considering all major phenomena photoproduction, photoinhibition and photo-regulation.

The correspondence between model and experimental data were analysed using algae cultivated in lab-scale PBRs simulating industrial cultivation conditions with high cellular density (1- 1.8 g L^{-1}) and inhomogeneous light distribution (supplementary figure S1, (Benvenuti et al., 2016; De Vree et al., 2015), see 2.1.2 for details). Nutrients and CO_2 were also provided in excess to ensure that algae growth was mostly dependent from their light to biomass conversion efficiency. Incident light intensity *I(0)* was set at 400 µmoles photons $m^{-2}s^{-1}$to saturate photosynthesis only in most exposed cells (Sforza et al., 2012b).

In order to predict cells growth in these conditions, the fluorescence model of (Bernardi et al., 2016) was calibrated using experimentally determined values of [Chl]/cell (expressed in pg Chl/cell), ASII (expressed as e^- per second), photosynthesis-irradiance (PI) curve and the fluorescence kinetic for the WT strains during cultivation in PBR. Figure 2 shows that the model is capable of representing accurately the experimental growth data of WT *N. gaditana* cultivated in the intensive conditions of a PBR . The model also correctly predicts that with the increase in biomass concentration ($x_{biom}(0)$) there is a progressive reduction in the specific growth rate as a consequence of the lower light intensity available per cell (Figure 2A) but also a slight increase in overall biomass productivity (Figure 2B), as experimentally observed (Sforza et al., 2015).

4.3 Prediction of the performances of hypothetical photosynthetic mutants in PBR.

Recent efforts of algae genetic engineering identified some modifications as potentially beneficial for productivity the reduction of Chl content per cell, decrease of antenna size (AS) and NPQ (Formighieri et al., 2012; Simionato et al., 2013; Wobbe et al., 2015). The model described above includes all these factors explicitly and quantitatively, making thus possible to predict *in silico* the effect of such modifications on productivity by modifying the values of the corresponding photosynthetic parameters (see 3.1 and 3.5).

To simulate a reduction in the activation of NPQ is indeed sufficient to reduce the values of the parameters $\bar{\eta}_{qE}^{F}$, $\bar{\eta}_{qE}^{S}$ and $\bar{\eta}_{qE}^{C}$, which represent the contribution of the different components to this regulatory process activated with different timescales (see equation 15, see 3.1 and 3.5 for details). The antenna size of PSII (ASII) is instead expressed as the ratio between σ/N, which are the chlorophyll specific total cross section of the photosystems and the number of chlorophylls associated with each reaction center, respectively. Therefore, in order to simulate a reduction of ASII, both σ and N have to be modified accordingly (see 3.1 and 3.5 for details).

Finally, given the parameter σ indeed depends on the self-shading between chlorophyll molecules, it is thus expected to be a decreasing function of the Chl content (see supplementary figure S2). Therefore, in order to simulate a reduction in the Chl content of the cells is sufficient to reduce the self-shading between chlorophylls and increase the value of σ (see 3.1 and 3.5 for details).

As shown in figure 3, according to the model predictions all suggested modifications are expected to provide a benefit in the specific growth rate with respect to the WT but the impact largely differs depending on the specific mutation. As example, the reduction in NPQ activation alone is predicted to have only a minimal effect (e.g. 0.5 % increase when NPQ_{mut} / NPQ_{WT} = 0.1, Figure 3A). This could be due to the fact that, in the considered growth conditions, the model predicts a very modest activation of NPQ, with a 10 % of maximal activation in the more external layers while this remains inactive in most of the culture (Figure 4) (in the model this activity is represented by the variables α_F and α_S, see 3.1 for details).

If a stronger illumination (e.g. *I(0) = 1500* μmoles photons $m^{-2}s^{-1}$) is applied to the culture it indeed induces a stronger NPQ activation (65 % of maximal activation in the more external layers, figure 4) but still NPQ is

not activated in most of the culture. Another factor to be considered is that the absence of this photo-protection mechanism impairs the cells ability to withstand intense illumination (Dall'Osto et al., 2005; Olivieri et al., 2015). Cells in external layers are in fact predicted to suffer from extensive photoinhibition, amplified by the inability of activating NPQ, reducing the potential advantages (Figure 5).

The reduction in ASII shows a larger positive impact with up to 14 % increase in the specific growth rate with a theoretical 50 % ASII reduction (Figure 3B). It is worth noting that such a reduction assumes a constant Chl content, and thus a smaller ASII triggers an increase in PSUs. The modification with the largest potential impact is however the reduction in Chl content that leads to more than a 30 % increase with respect to the WT with a ≈ 60 % reduction (Figure 3C). It is interesting to point out that the model also predicts that Chl reduction below this limit becomes actually detrimental and causes a decrease in the productivity. This reduction can be attributed to the fact that algae culture become so transparent that a significant fraction of incident light is not absorbed (25 % according to the model prediction, see figure 6).

4.4 Validation of the model with experimental data of Nannochloropsis gaditana photosynthetic mutants.

As shown above the mathematical model developed is capable of predicting the impact of different modifications of the photosynthetic apparatus on productivity. It can also provide substantial additional information such as the extent of the light induced damage or the illumination available in the different parts of the culture (Figure 5A-B and 6B) that are extremely or impossible to assess experimentally.

The model prediction ability was validated experimentally using three available *N. gaditana* photosynthetic mutants selected as the most promising after the screening of a random collection (Perin et al., 2015). As shown in Table 1, these mutants show variable alterations in the photosynthetic parameters here considered, since NPQ, ASII and Chl content are not completely independent one from the other.

As shown in Table 1, I48 shows the largest reduction (95 %) in NPQ with respect to the WT strain and a smaller reduction, 5 and 15 %, in both ASII and Chl content, respectively. On the contrary, strain E2 shows a larger difference in ASII and Chl content and a more contained reduction in NPQ (50 %). Finally the third

mutant, I29, shows instead an unaltered NPQ and the largest reduction in both ASII and Chl content (Table 1). All three strains also showed an increased biomass productivity and specific growth rate with respect to the parental strain during the cultivation in PBR in the same conditions used for WT above (Table 1 and supplementary figure S3).

Experimental productivities were thus compared with model predictions for strains showing specific alterations in photosynthetic properties. As shown in figure 7 there is a very good fit for both E2 and I48 strains, while the increase in both biomass productivity and the specific growth rate for I29 deviates from the model prediction. Measured productivities also fully confirmed the model prediction that alterations in NPQ activation have limited effects on *N. gaditana* productivity, at least for growth conditions tested in this work (Figure 3A). On the other hand, the reduction in Chl content confirmed to have the largest impact on productivity in the tested conditions (Figure 7). I29 indeed showed the largest reduction in both ASII and Chl content with respect to the WT strain (Table 1) and also the best performances (Figure 7, Supplementary figure S4).

The discrepancy between measured and predicted values in the case of I29 is likely due to the fact that the mutant shows a decrease in cells size with respect to WT that was not accounted for in model predictions. While cells size is a model parameter, it also affects indirectly other model parameters such as σ, Chl content and cell maintenance (M) and in present form it is not possible to predict its overall effect on productivity (Bišová and Zachleder, 2014).

It is worth noting that the model was calibrated only with data obtained from the WT strain cultivated in PBR and no information from the mutants was exploited for predictions. The presented model is thus able to predict the growth phenotype of modified algal strains before these are characterized and has the potential of drastically reducing the time and experimental costs to achieve concrete improvements in the field of algae genetic engineering by predicting the optimal genotype to be generated. The possibility of using a model in such a tailored way is granted by the fact that model was identified in rigorous way, with all parameters estimated with statistical significance (Bernardi et al., 2016). In other words, the model

parameters are not simply tuned to fit the data, but retain their physical significance in the model equations.

5. DISCUSSION

In this work, we developed a quantitative model to predict microalgae growth during intensive cultivation in a PBR, combining a model describing photosynthetic productivity as function of the available light (Bernardi et al., 2016) with the mathematical description of the light attenuation profile in a microalgae dense culture. The model was shown to be able to describe quantitatively microalgae growth in such environment and the influence of different light supply rates (Figure 2). It can be concluded that the presented model thus accounts for all major parameters influencing microalgae growth in a PBR (see section 3 for details).

This model was then exploited to simulate *in silico* alterations in key photosynthetic parameters and predict their effect on algae growth in PBR. We assumed hypothetical reductions in ASII, Chl content and NPQ and observed that such alterations were predicted to have an overall positive effect on growth of microalgae cells (Figure 3), as suggested by recent literature in the field (Berteotti et al., 2016; Cazzaniga et al., 2014; Kirst et al., 2014). Model predictions suggested that specific modifications have however a largely different impact with NPQ alone showing a negligible effect, while the modulation of Chl content was identified as the most promising target of genetic engineering (Figure 3). The reliability of the model predictions was tested with three available photosynthetic mutants showing variable combinations of modifications of the photosynthetic apparatus, obtaining a satisfactory correspondence with experimental data (Figure 7). The developed model is thus indeed able to predict the effect of genetic modifications of the photosynthetic apparatus on algae productivity in PBR with a good reliability. In one case, the difference between the model prediction and experimental data was larger, likely because the mutation also caused an alteration in cells size that was not included in the model predictions. The model clearly can only predict phenomena that are represented through its equations and parameters; if a genetic modification affects a feature that was not taken into account, it is expected a loss in accuracy. On the other hand, the satisfactory fit between

predictions and experimental data suggests that major phenomena affecting productivity are mathematically described and included and that the model can be exploited to reliably predict the performances of a modified strain in a PBR context reducing the need for time consuming larger scale validations. It is worth underlying that we exploited a model whose parameters have a physical significance, allowing an *a priori* prediction of how improved strains should behave when tested in an industrially relevant environment. Such an ability to predict the effect of a panel of photosynthetic modifications on growth performances can be highly valuable to direct genetic engineering efforts *a posteriori* towards the biological targets showing the greatest impact on productivity. Moreover, this model was identified in a rigorous way, according to state-of-the-art techniques. The presented model clearly suggests that the Chl content is the most impactful parameter and this, in combination with a reduction in antenna size, has the potential to provide a maximum 43 % increase in specific growth rate, assuming the best theoretical combination of alterations for these parameters, namely a 100 %, 60 % and 50 % reduction in NPQ activation, Chl content and ASII, respectively, see supplementary figure S5). This model could thus make the whole process of isolation of improved strains much more efficient, reducing experimental time and costs. It is also worth underlining that once proved its applicability, such a model can be also applied to tailor the genetic modifications to reach the best productivities depending on the cultivation system used (e.g pond vs. PBR, semi-continuous vs continuous cultivation etc.) or on the environmental conditions at the installation site (e.g tropical or temperate areas). An example of the predictive potentiality of this model is shown in figure 8 where the productivity predictions of *Nannochloropsis gaditana* WT and the mutant E2 are reported showing the dependence from the season (January or July), the cultivation sites (The Netherlands, Italy or Gibraltar) and PBRs geometry (5 or 10 cm diameter). In the longer term, such model will be implemented to provide specific answers for each cultivation condition and will open the possibility of generating strains tailored to a specific cultivation site.

It is also important to point out the limitations of the model, due to its construction. First of all, this model assumes that growth depends strictly from the available light, that is a correct assumption only when

nutrients and CO_2 are provided in excess to the cultures as done here. Another possible limitation of the current model is that it describes algae growth in a stable cultivation environment where cells indeed show a unique photosynthetic acclimation state and it does not include the effects of a dynamic light environment. The latter is indeed expected to impact the performances of improved photosynthetic strains, as previously demonstrated in cyanobacteria (Kirst et al., 2014; Page et al., 2012) and in algae as well (Perin et al., submitted). A complete description of photo-acclimation and its influence on productivity will clearly make the model more complex (Bernardi et al., 2017; García-Camacho et al., 2012) but it will also increase accurancy in predictions of outdoor cultivation.The presented model could be integrated with simplified metabolic modelling for instance accounting for different biomass components, in order to evaluate as example lipids productivity and not only total biomass. Finally, it is also important to underline that here model parameters were estimated using experimental data collected from *Nannochloropsis gaditana* WT cells, but model equations are general and do not depend on the species. The same model after re-parametrization with new experimental data could thus be applied to predict the growth in PBR of other algae species and consequently the effect on growth of modifications of their photosynthetic metabolism.

6. CONCLUSIONS

In this work, we developed an empirical light attenuation model to describe the light profile inside high density algae cultures. By combining this model with the mathematical description of algae photosynthetic productivity as function of available light, we predicted the growth of *Nannochloropsis gaditana* during cultivation in PBR. By changing the values of key photosynthetic parameters of the model is was possible to predict the growth of strains with altered photosynthetic phenotypes. Model predictions were validated with data from photosynthetic mutants, demonstrating for the first time that this tool can be exploited to assess the response and the effectiveness of genetic engineering efforts, directing genetic engineering *a posteriori* towards those photosynthetic targets showing the highest impact on biomass productivity, in an industrially relevant environment.

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AUTHORS' CONTRIBUTIONS

TM and FB designed the research; GP and ABellan performed the photosynthesis measurements and growth monitoring in PBR; ABernardi and FB built the mathematical models; GP and ABernardi analyzed data; GP and TM wrote the manuscript. All authors read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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FIGURES

Figure 1. Light attenuation model in PBR. Measured (squares) and model predictions (solid red line) values of $K_{LB}(x_{biom})$ for *Nannochloropsis gaditana* wild type strain in a PBR. The values of the hyperbolic model parameters are K_{LB}^{max} = 296 and k_B = 248. (color, 1-column fitting image)

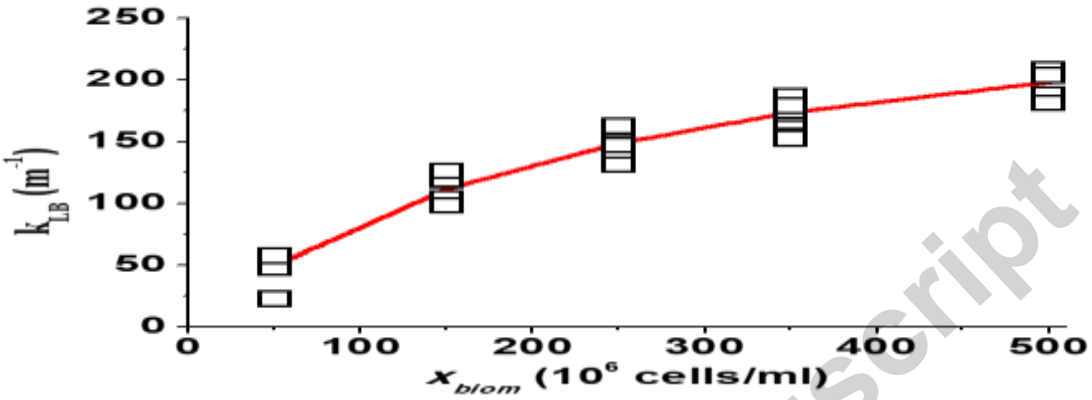

Figure 2. Growth of *Nannochloropsis gaditana* wild type strain in PBR. Squares represent the experimentally measured growth rate constants and biomass productivity values, respectively in A and in B. In both panels, the red solid line represents the growth profile predicted by the model developed. Error bars for the prediction of the model represent the estimated uncertainty of both σ and τ parameters (see 3.4 and 3.5). The experimental growth data here reported were collected during WT cultivation in PBR operated in semi-continuous mode, as described in section 2.1.2. (color, 2-columns fitting image).

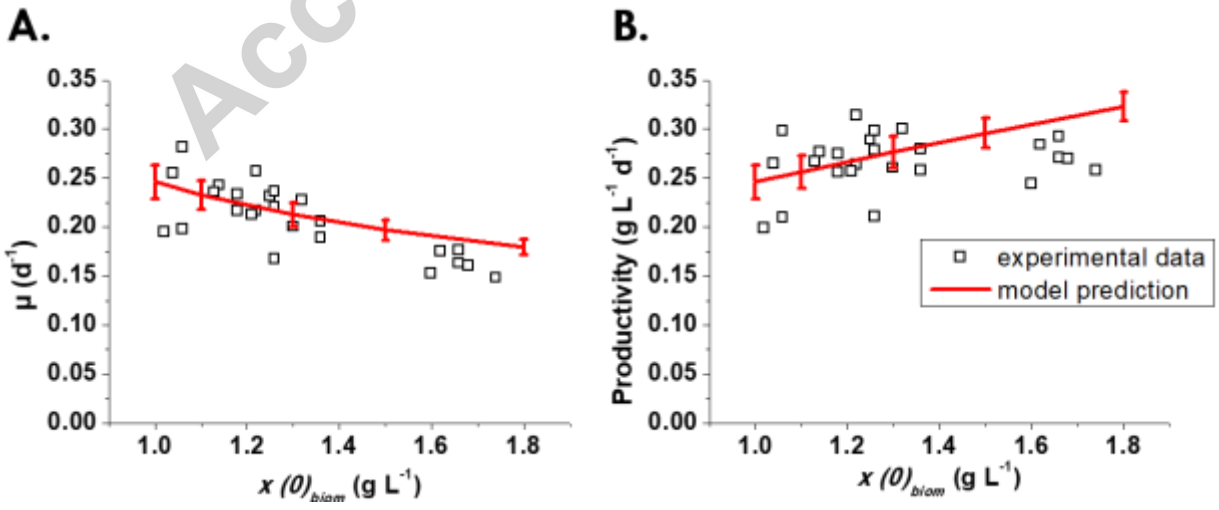

Figure 3. Growth prediction for *Nannochloropsis gaditana* photosynthetic mutants. The effect of modifications in the NPQ, ASII and Chl content on algae cultures productivity is shown in A, B and C, respectively. The increase in the specific growth rate of the hypothetical photosynthetic mutants (squares and solid lines) is here expressed as percentage with respect to the WT strain. Error bars for the predictions of the model come from the uncertainty of both σ and τ parameters (see 3.4 and 3.5). A maximal reduction of 50 % in the ASII was considered, since a significant fraction of the pigments are bound to the reaction centers and are thus not removable (Melis, 1991). The predicted growth rate increase was calculated considering a starting biomass concentration ($x_{biom}(0)$) of 1.3 g L^{-1} for the WT strain, a median value of those concentrations considered in figure 2 (color, 1.5-column fitting image).

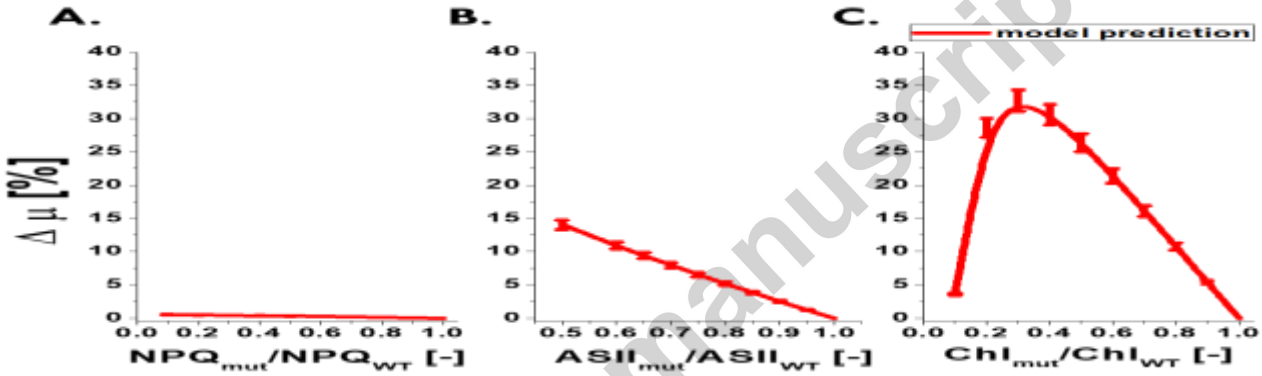

Figure 4. Model prediction of the NPQ activation within a *Nannochloropsis gaditana* culture in a PBR. The prediction of NPQ activation is here expressed as percentage of its maximal activity for *Nannochloropsis gaditana* WT strain, as a function of the depth (in centimeters, cm) of the PBR. Two profiles of NPQ activation are here indicated, as a consequence of the light intensity reaching the culture, respectively 400 (black curve) or 1500 (red curve) µmoles photons $m^{-2}s^{-1}$. (color, 1-column fitting image).

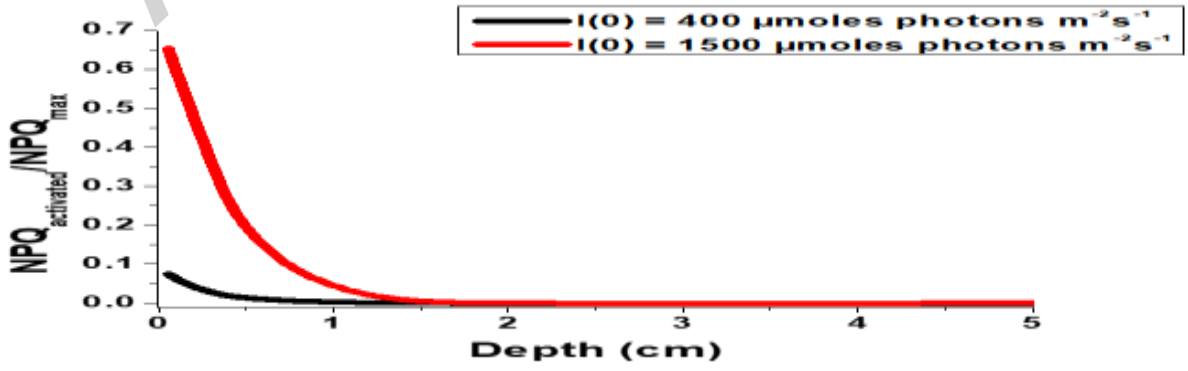

Figure 5. Model prediction of the photoinhibition levels in a *Nannochloropsis gaditana* culture in PBR. The prediction of the number of inhibited PSUs is here expressed as fraction of the total PSUs for *Nannochloropsis gaditana*, as a function of the depth (in centimeters, cm) of the PBR. The fraction of inhibited PSUs is here reported for cultures illuminated with 400 or 1500 µmoles photons $m^{-2}s^{-1}$, respectively in A and in B. Two profiles of fractions of inhibited PSUs are indicated in both panels, the green one referring to WT, while the blue one to an ideal mutant unable to activate NPQ. (color, 2-columns fitting image).

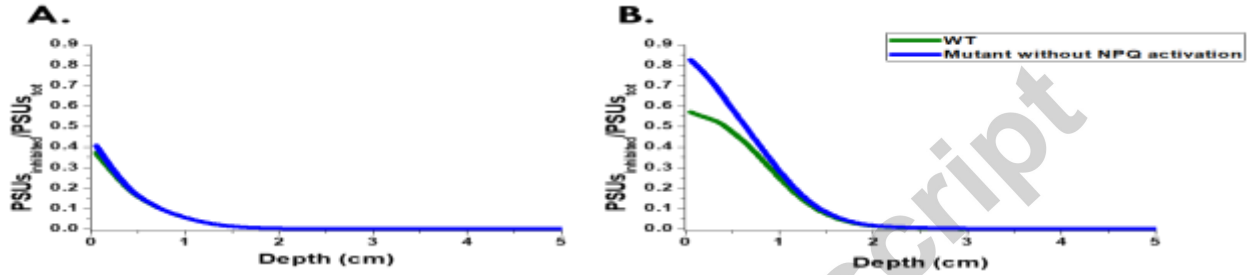

Figure 6. Model prediction of the light profile in the PBR as a function of the Chl content of the *Nannochloropsis gaditana* cells. The prediction of the irradiance reaching different culture layers and the fraction of the inhibited PSUs is expressed as a function of the depth (in centimeters, cm) of the PBR, respectively in A and B. Both panels show different profiles of irradiance or inhibited PSUs, according to the increasing reduction in the Chl content of mutant cells; Chl_{mut}/Chl_{WT} = 1 in black, Chl_{mut}/Chl_{WT} = 0.5 in red, Chl_{mut}/Chl_{WT} = 0.1 in blu. (color, 2-columns fitting image).

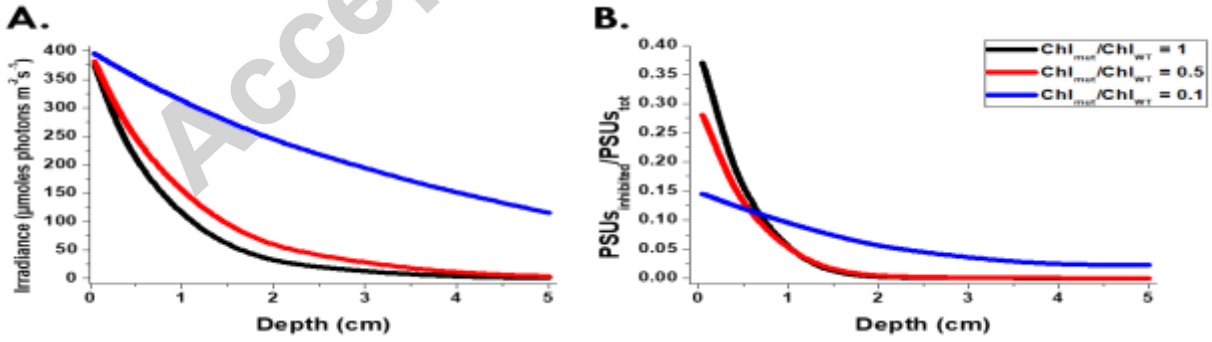

Figure 7. Experimental validation of model ability to predict effect of genetic modifications. The model prediction for the increase in biomass productivity (A) and in the specific growth rate (B) of selected *N. gaditana* photosynthetic mutants, expressed as percentage with respect to the WT, is here depicted by the

histograms. Error bars for the predictions of the model come from the uncertainty of both σ and τ parameters (see 3.4 and 3.5). Solid squares instead represent the corresponding experimentally measured values for the three photosynthetic mutants available, when cultivated in PBR, according to the conditions chosen in this work (see 2.1.2 for details). Experimental measured data belong to a population with n > 40. The predicted increases were calculated considering as starting biomass concentration ($x_{biom}(0)$) for the WT strain, the average of the experimentally measured values (1.3 g/L). The whole model prediction profile for the specific growth rate is reported in supplementary figure S4) (color, 2-columns fitting image)

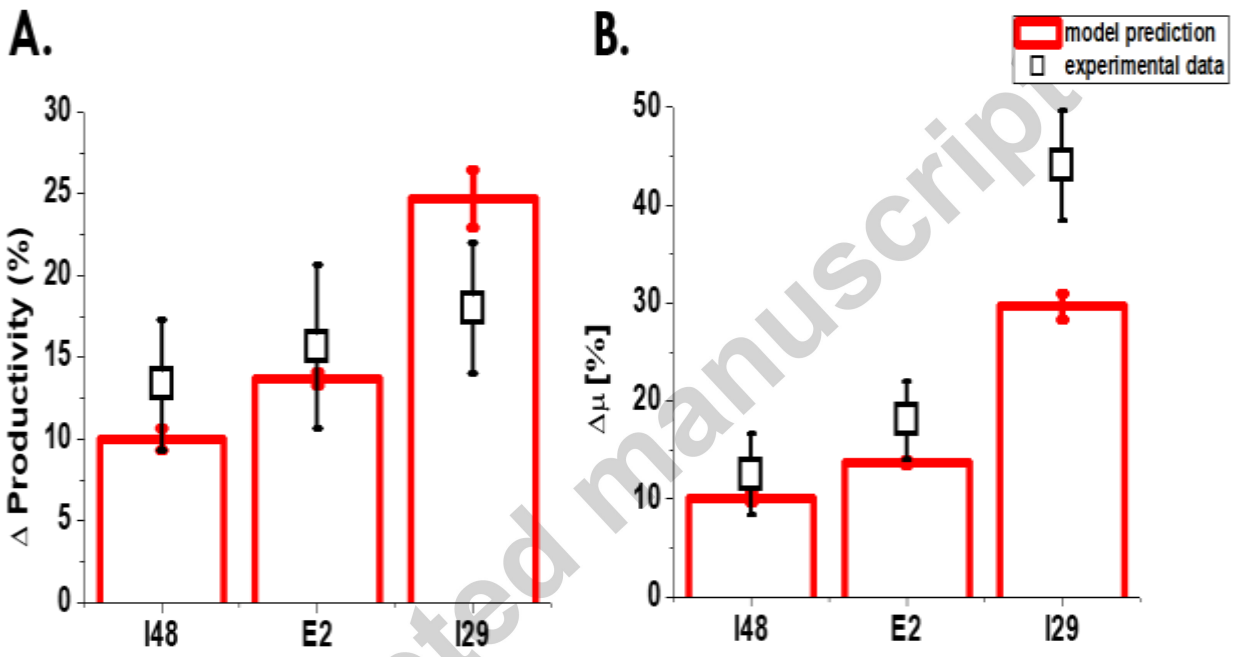

Figure 8. Biomass productivity predictions in different industrial sites, seasons and PBR geometries. The average biomass productivity (g L^{-1} d^{-1}) predictions during a day of outdoor cultivation of both *Nannochloropsis gaditana* WT strain (black) and a photosynthetic mutant (E2, red), are here represented as a function of the cultivation site (A,D,G The Nethederlands; B, E, H Padova (Italy) and C,F, I Gibraltar) and season (A, B, C January and D-I July), in a cylindrical PBR with 5 cm (A-F) or 10 (G-I) diameter. For the specific irradiation profile (grey area in each graph) we referred to the following website: http://re.jrc.ec.europa.eu/pvgis/apps4/pvest.php#. Latitude: 52.4 (The Netherlands); 45.4 (Padova); 36.1 (Gibraltar). Longitude: 4.9 (The Netherlands); 11.9 (Padova); -5.3 (Gibraltar).

Despite a deeper PBR is predicted to produce less biomass per unit of volume, the final amount of biomass obtained per day is higher as a consequence of its higher volume (see Supplementary Table S3). (2-columns fitting image)

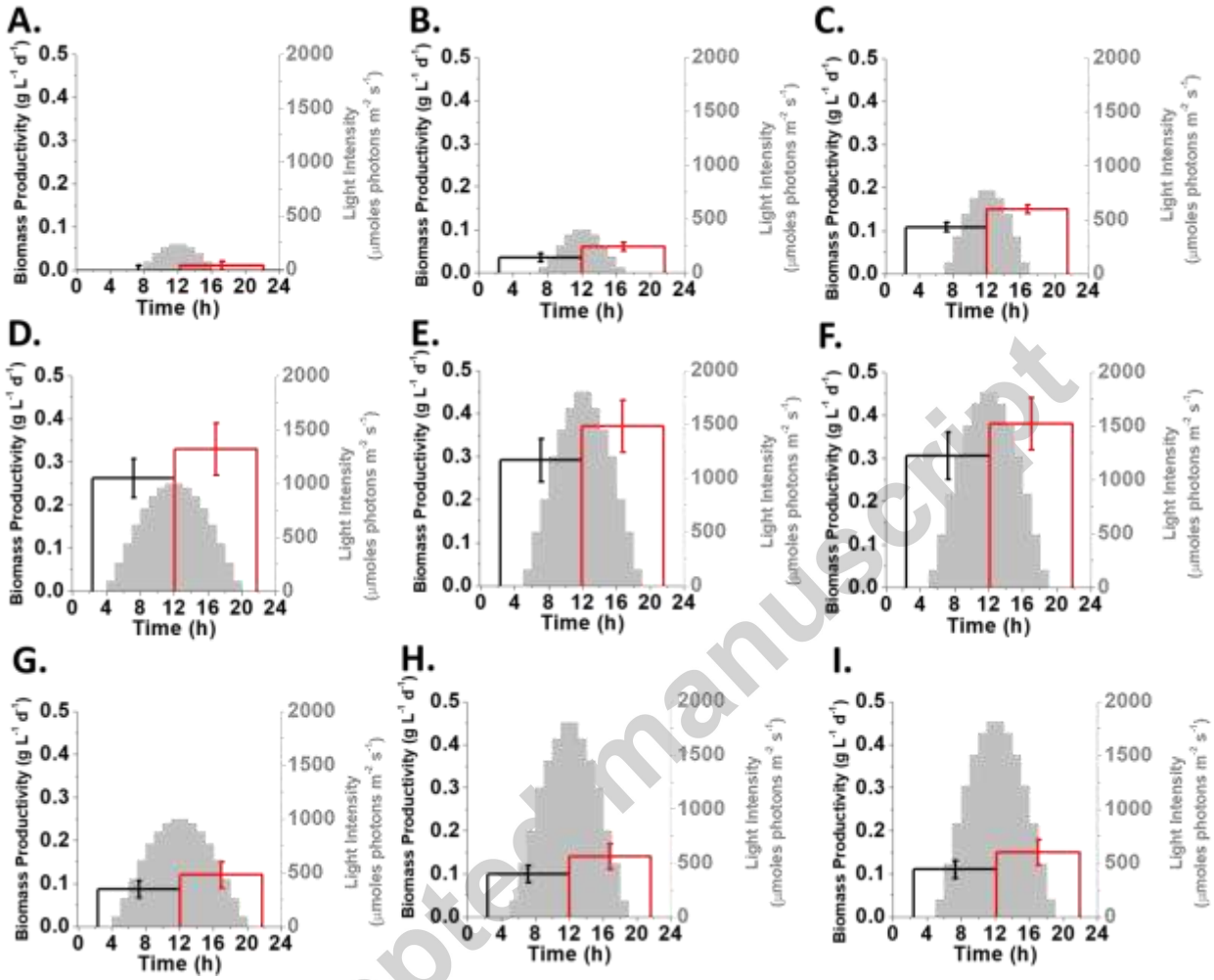

Table 1. Photosynthetic properties and growth of selected *Nannochloropsis gaditana* mutant strains in PBR. The photosynthesis and growth data here reported were determined experimentally for microalgae cultivated in PBR operated in semi-continuous mode, as described in section 2.1.2. All data are here represented relatively to WT strain, while all experimentally determined values are shown in supplementary figure S3. All these data indicate statistically significant differences between mutants and

parental strain (One-way ANOVA, p-value < 0.05); n > 20 for photosynthetic data; n > 40 for growth data.

(no color, 2-columns fitting table)

Strain	NPQ_{mut}/NPQ_{WT}	$ASII_{mut}/ASII_{WT}$	Chl_{mut}/Chl_{WT}	$Productivity_{mut}/Productivity_{WT}$	μ_{mut}/μ_{WT}
I48	0.05	0.95	0.85	1.13 ± 0.05	1.12 ± 0.04
E2	0.5	0.9	0.8	1.15 ± 0.06	1.18 ± 0.04
I29	1	0.75	0.57	1.17 ± 0.05	1.44 ± 0.05

SUPPLEMENTARY MATERIAL

Supplementary Table S1. Summary of all model variables and parameters including unit of measure.

Supplementary Table S2. Values of $I^w(h)$ and $I^{WT}(h, x_{biom})$ measured for the *Nannochloropsis gaditana* WT and mutant strains I48 and E2.

Supplementary Table S3. Total biomass production predictions in PBRs with different depth.

Supplementary figure S1. Schematic representation of the lab-scale PBR used in this work.

Supplementary figure S2. Estimated ratio between σ in *Nannochloropsis gaditana* mutants showing a reduction in both Chl content and ASII in PBR and the value of the wild type strain.

Supplementary figure S3. Experimental data collected for all the *Nannochloropsis gaditana* strains exploited in this work.

Supplementary figure S4. Model validation.

Supplementary Figure S5. Model prediction of the greatest improvement in growth achievable in *Nannochloropsis gaditana*.

HIGHLIGHTS

- A mathematical model to predict algae growth in a photobioreactor was developed;
- The model predicts effects of alterations in photosynthetic metabolism;
- Model predictions were validated with experimental data from mutated strains;
- The model can identify priority targets for genetic engineering of photosynthesis