Journal of Biotechnology 259 (2017) 63–72

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Semi-empirical modeling of microalgae photosynthesis in different acclimation states – Application to *N. gaditana*

Andrea Bernardi[a], Andreas Nikolaou[b], Andrea Meneghesso[c], Benoît Chachuat[b], Tomas Morosinotto[c], Fabrizio Bezzo[a,*]

[a] CAPE-Lab (Computer-Aided Process Engineering Laboratory) and PAR-Lab (Padova Algae Research Laboratory), Department of Industrial Engineering, University of Padova, Italy
[b] CPSE (Centre for Process Systems Engineering), Department of Chemical Engineering, Imperial College London, UK
[c] PAR-Lab (Padova Algae Research Laboratory), Department of Biology, University of Padova, Italy

ARTICLE INFO

Keywords:
Microalgae
Photoacclimation
PI curves
Nannochloropsis gaditana
Non-photochemical quenching

ABSTRACT

The development of mathematical models capable of accurate predictions of the photosynthetic productivity of microalgae under variable light conditions is paramount to the development of large-scale production systems. The process of photoacclimation is particularly important in outdoor cultivation systems, whereby seasonal variation of the light irradiance can greatly influence microalgae growth. This paper presents a dynamic model that captures the effect of photoacclimation on the photosynthetic production. It builds upon an existing semi-empirical model describing the processes of photoproduction, photoregulation and photoinhibition via the introduction of acclimation rules for key parameters. The model is calibrated against a dataset comprising pulsed amplitude modulation fluorescence, photosynthesis rate, and antenna size measurements for the microalga *Nannochloropsis gaditana* in several acclimation states. It is shown that the calibrated model is capable of accurate predictions of fluorescence and respirometry data, both in interpolation and in extrapolation.

1. Introduction

By virtue of a much higher areal productivity compared to conventional terrestrial crops and a reduced impact on food production, microalgae constitute a promising feedstock for the sustainable production of biomaterials, high-value biochemicals, and even biofuels (Leu and Boussiba, 2014; Georgianna and Mayfield, 2012). Nonetheless, microalgae cultivation remains expensive and the light conversion efficiency obtained in large-scale production systems are far from their theoretical limits. For instance, carotenoids and polyunsaturated fatty acids (PUFA) extracted from microalgae remains 3–5 times more expensive than the current market prices (Leu and Boussiba, 2014). For biodiesel production likewise, a decrease in the production costs by a factor of 10 would be necessary to achieve economic profitability (Wijffels and Barbosa, 2010).

In this context, the development of reliable mathematical models, capable of quantitative predictions of microalgae growth are invaluable tools to unveil the biological and physical mechanisms at play and, in turn, develop an improved, or de-bottleneck an existing, production system using systematic optimisation techniques (Bernard et al., 2016). However, the development of such models for large-scale systems faces numerous challenges due to numerous competing biological processes that span multiple time- and space-scales. Among the models available in the literature, the so-called state models (Han et al., 2000; Wu and Merchuk, 2001) based on the concept of photosynthetic unit (PSU) have been successful at representing industrial production systems. However, they typically capture a limited number of key photosynthetic mechanisms and their interactions, and they need tuning to describe the macroscopic behaviour of a culture in a given set-up, which renders extrapolation risky in the presence of numerous unknown parameters.

As far as light-driven photosynthesis is concerned, four main biological processes acting on different time scales are usually distinguished (Williams and Laurens, 2010): *photoproduction* encompasses all the processes from photons utilisation to CO_2 fixation that occurs within milliseconds; *photoregulation*, also known as non-photochemical quenching (NPQ), is the set of mechanisms by which microalgae protect their photosynthetic apparatus via the dissipation of excess energy into heat, and occurs within minutes; *photoinhibition*, the observed loss of photosynthetic production due to excess or prolonged exposure to light, takes place on a time scale of minutes to hours; and *photoacclimation* entails a reduction of the cell chlorophyll content and an increase in photoprotective xanthophylls in response to long-term light variations (Rodriguez et al., 2006).

* Corresponding author.
E-mail address: fabrizio.bezzo@unipd.it (F. Bezzo).

http://dx.doi.org/10.1016/j.jbiotec.2017.08.002
Received 11 January 2017; Accepted 1 August 2017
Available online 12 August 2017
0168-1656/

Recently, we developed a state model describing the dynamics of photoproduction, photoregulation and photoinhibition. We calibrated this model using pulsed amplitude modulation (PAM) fluorometry and photosynthesis rate (PI) lab-scale measurements (Nikolaou et al., 2015; Bernardi et al., 2016) for the microalga *N. gaditana*, and established that it is capable of accurate predictions under complex light patterns. Early attempts to combine this state model with imperfect mixing and light attenuation effects into multi-physics models suggest that the interactions between the biological and physical processes can explain the productivity losses observed in large-scale microalgae production systems (Nikolaou et al., 2016). In order to make a more definitive assessment and enable the application of systematic optimisation methods, photoacclimation processes should be accounted for in this multi-physics model.

The main objective of this paper is to introduce the slower process of photoacclimation into this modelling framework in order to improve the overall predictive capability and applicability of the model in Bernardi et al. (2016). Although a number of mathematical models accounting for the slower processes of photoacclimation and nitrogen limitation are already available in the literature (Geider et al., 1998; Bernard, 2011), they do not consider the faster processes of photoregulation and photoinhibition. Likewise, the model by Garcia-Camacho et al. (2012), inspired from Zonneveld (1998), describes photoinhibition and photoacclimation under nitrogen replete conditions, but it does not account for photoregulation. In contrast, this paper presents a semi-empirical model that includes the faster processes of photoproduction, photoregulation and photoinhibition altogether, and enables predictions in different acclimation states at the same time. An important assumption supporting this modelling is that the dynamics of photoacclimation are slow compared to those of photoproduction, photoinhibition and photoregulation, and therefore the acclimation state can be considered constant on time scales of up to several hours. The emphasis in developing the acclimation rules is on keeping the model as simple as possible, and in particular keeping the number of parameters to a minimum. Such a compromise between simplicity and reliability is key to enabling applications in monitoring, control and optimisation of microalgae production systems. Building upon our previous model (Bernardi et al., 2016) presents the additional benefit that information rich fluorescence signals from a PAM fluorometer can be used in combination with PI measurements for calibration purposes.

The remainder of the paper is organised as follows. Section 2 presents the experimental measurements used to develop, calibrate and validate the proposed model. A semi-empirical relationship describing the cell chlorophyll content as a function of the acclimation state is introduced in Section 3.1. The acclimation rules for photoproduction and photoregulation expressing a subset of the parameters of the fluorescence model developed in Nikolaou et al. (2015), Bernardi et al. (2016) (and reported in Appendix A) as a function of the acclimation state are presented in Sections 3.2. Additional relationships between the model variables and parameters and the experimental measurements are discussed in Section 3.3. The calibration and validation results are presented and discussed in Section 4. Finally, Section 5 concludes the paper.

2. Materials and methods

The microalga *Nannochloropsis gaditana* (CCAP, strain 849/5) was grown in a sterile, filtered F/2 medium, using sea salts (32 g L^{-1}) from Sigma, 40 mM Tris–HCl, pH 8 and Sigma Guillard's (F/2) marine water enrichment solution. Growth experiments were performed in the multicultivator MC 1000-OD system (Photon Systems Instruments, Czech Republic) at a temperature of 21 °C. The suspension culture was constantly mixed and aerated by bubbling air. Pre-cultures were grown at 100 μE m^{-2} s^{-1} in glass bottles of 0.25 L under a continuous airflow, enriched with 5% CO_2. At the exponential phase, the pre-culture was centrifuged and re-suspended in fresh medium to reach a final concentration of 9×10^6 cells mL^{-1}, before introduction in a lab-scale photobioreactor ("multicultivator" from PSI – Photon Systems Instruments). 11 growth experiments were performed using different light intensities provided continuously by an array of white LEDs; the set of light intensities is: 10, 30, 50, 70, 100, 150, 200, 400, 700, 750, 1000 μE m^{-2} s^{-1}. Samples from the different cultures were used to perform four types of measurements, which have been used for model calibration and validation and are briefly described next.

The cell chlorophyll content was measured for all 11 acclimation states. Chlorophyll was directly extracted from intact cells using 100% N,N-dimethylformamide (DMF) (Sigma-Aldrich, Italy) for at least 48 h in the dark at 4 °C after centrifugation of Nannochloropsis cells at 15000 × *g* for 10 min (Meneghesso et al., 2016). Pigment concentrations were determined spectrophotometrically using a Cary 1000 spectrophotometer (Agilent Technologies, USA).

The fluorescence fluxes emitted by the chlorophyll molecules were measured for 8 acclimation states, using a dual PAM (Walz, Germany). Various amounts of cells were put into the cuvette in order for the amount of chlorophyll to be between 5 and 20 μg in each PAM experiment: 87, 116, 86, 173, 193, 251, 178 and 250 [10^6 cells] for the samples acclimated at 10, 30, 50, 70, 100, 400, 700 and 1000 μE m^{-2} s^{-1}, respectively. Following a dark adaptation period of 20 min, increasing actinic light intensities were applied in steps of 60 s. Before switching-on of the actinic light and during the final 2 s of each step, a saturating light pulse of 6000 μE m^{-2} s^{-1} was applied during 0.6 s, followed by a dark period (actinic light off) of 1.4 s; measurements were recorded before and after the saturating pulses and after the dark periods, which correspond to the realised (F'), maximum (F'_m) and minimum (F'_0) fluorescence fluxes, respectively.

Photosynthesis rates were measured for 7 acclimation states. Out of the possible ways of measuring the photosynthesis rate, we considered the maximal rate of photosynthetic oxygen evolution at a specific actinic light using a Clark-type O_2 electrode (Hansatech, UK). We performed three separate experiments for each acclimation state, during which the microalgae samples were exposed to two different light intensities for a variable amount of time, thus providing a total of 6 experimental points. The first sample was exposed to 100 μE m^{-2} s^{-1} for 230 s and at 700 μE m^{-2} s^{-1} for 200 s; the second sample was exposed to 400 μE m^{-2} s^{-1} for 130 s and to 1500 μE m^{-2} s^{-1} for 150 s; and the third sample was exposed to 250 μE m^{-2} s^{-1} for 150 s and to 3600 μE m^{-2} s^{-1} for 130 s. The photosynthesis rate was measured at the end of each time period.

Finally, the antenna size of photosystem II (functional antenna size (ASII)) was measured for 5 acclimation states, namely 10, 70, 400, 700 and 1000 μE m^{-2} s^{-1}. A LED pump-probe JTS-10 spectrometer in fluorescence mode was used for the measurements, following a dark-adaptation period of 20 min. Fluorescence inductions were measured in the infrared region of the spectrum upon excitation with blue light at 450 nm. During dark adaptation, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added at a concentration of 80 μM to prevent oxidation of the primary quinone acceptor Q_A (Meneghesso et al., 2016; Simionato et al., 2013). In the presence of such an inhibitor, ASII is inversely proportional to the half-saturation time constant of the fluorescence response rise-time (Bonente et al., 2012). The antenna size experiments were conducted for five different actinic light intensities (45, 80, 150, 320 and 940 μE m^{-2} s^{-1}) at 630 nm, and four replicates were measured at each light intensity.

3. Model development

The proposed model builds upon the chlorophyll fluorescence model developed in Nikolaou et al. (2015) and Bernardi et al. (2016), whose complete set of equations is reported in Appendix A for the sake of completeness. Following Bernard (2011), the acclimation state is represented by the light intensity I_g at which the culture was grown. A