Algal Research 33 (2018) 430–439

Contents lists available at ScienceDirect

Algal Research

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

Modelling the photosynthetic electron transport chain in *Nannochloropsis gaditana* via exploitation of absorbance data

Riccardo De-Luca[a], Andrea Bernardi[a], Andrea Meneghesso[b], Tomas Morosinotto[b], 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, via Marzolo 9, 35131 Padova, Italy
[b] PAR-Lab (Padova Algae Research Laboratory), Department of Biology, University of Padova, Via Ugo Bassi 58/B, 35121 Padova, Italy

ARTICLE INFO

Keywords:
Microalgae
Photosystem I
Nannochloropsis gaditana
Mathematical modelling

ABSTRACT

The development of mathematical models describing the photosynthetic apparatus of microalgae is paramount to gain deeper knowledge of the involved biological process and enable optimisation of cultivation conditions. This paper presents a dynamic model of the entire photosynthetic apparatus including the photosystems I (PSI) and II (PSII), the electron carriers between the two photosystems (plastoquinone, cytochrome b6f and cytochrome c6) and the final electron acceptor complex, the ferredoxin. In vivo measurements of PSI oxidation dynamics at different light intensities for the microalga *Nannochloropsis gaditana* have been exploited to develop and calibrate the model. The model has been experimentally identified and proved to be capable of accurate predictions of both linear and cyclic electron flows dependence on light intensity.

1. Introduction

Microalgae constitute a promising feedstock for the sustainable production of biomaterials, high-value biochemicals and biofuels thanks to a much higher areal productivity compared to terrestrial crops [1,2]. A major advantage is also that, unlike higher plants, over 90% of the microalgae biomass can be processed into food, feed, chemicals or energy, thereby supporting the development of microalgae biorefineries. Despite the great potential as renewable feedstock, microalgae production is still too expensive and a reduction of 5 to 10 times of the production costs is necessary for microalgae to be competitive with the current alternatives [2,3]. In order to achieve this goal one of the main issues to address is bridging the gap between maximal theoretical productivity and practical biomass productivity in large scale cultivation systems. Microalgae, in fact, can potentially convert 13% of the total solar light energy into chemical energy through photosynthesis [4]. Nevertheless, microalgae cultivation in artificial systems dramatically reduces the actual light-to-energy conversion to 1% or lower depending on the chosen cultivation system [5].

In this context, a quantitative description through robust and reliable mathematical models of key phenomena affecting microalgae growth, such as light utilization, is paramount to deepen our knowledge and enable process optimisation.

The series of reactions of the so-called light phase of the photosynthesis occur in the photosynthetic transport chain, i.e. the set of macromolecules responsible for the conversion of the light energy into chemical energy and of the water hydrolysis. Two reaction centers are responsible of the conversion of the light into chemical energy: photosystem II (PSII), where the absorbed light energy is exploited to split the water into oxygen and protons, and the photosystem I (PSI), which uses the light energy to reduce ferrodoxin. Final result of these electron transport reactions is the synthesis of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). Between the two photosystems a series of macromolecules, usually referred as electron carriers, are responsible for the electron transport from PSII to PSI.

In the literature several mathematical models have been proposed over the recent years (see [6] and [7] for a review). However they all focus on PSII, as its activity is strongly correlated with the linear electron transport from water to NADPH. A further major reason is that PSII activity can be investigated through in vivo fluorescence measurements, which are simple to perform. In this context, Nikolaou et al. [8] and Bernardi et al. [9] have recently proposed a semi-mechanistic model (based on Han 'state-model' approach [10]) to link three distinct processes acting on PSII at different time scales (photoproduction, photoinhibition and photoregulation) to the PSII fluorescence fluxes. They showed how considering chlorophyll-a fluorescence dynamics may help providing reliable predictions of the photosynthetic

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

https://doi.org/10.1016/j.algal.2018.06.016
Received 13 November 2017; Received in revised form 11 June 2018; Accepted 16 June 2018

response under variable light conditions, thus allowing for key photosynthetic mechanisms mathematical modelling, and leading to a rather comprehensive description of the PSII dynamics depending from different illumination intensities.

On the other hand, a lower number of models describing the entire electron transport chain has been proposed, and thus biologically relevant processes where PSII is not directly involved, such as cyclic electron flow [11], are not being considered. This restriction limits the potential applicability of these models to the environmental conditions where non-linear electron transport is playing a significant role, such as nutrients deficiency [12]. The available models describing the entire electron transport chain, recently reviewed by [13], are an efficient mean for numerical analysis of the electron and proton transport in the chloroplasts, but are usually extremely complex and thus difficult to be identified and validated (see for instance [14] and [15]). In fact, it is important to note that the observability of the system is low, as intermediate electron transport reactions are difficult to monitor in vivo, reducing the number of experimental data available for model calibration. The aim of this paper is to face these limitations and develop an electron transport chain model to provide an accurate representation of experimental data, yet retaining a simple structure to assure the precise identification of the model parameters. The model by [9] has been retained to describe PSII, including the values of its parameters as estimated using fluorescence measurements and additional semi-mechanistic dynamic equations have been introduced to describe the electron carriers and the PSI oxidation state. In vivo absorbance measurements have been exploited for estimation of total and cyclic electron flow required for model calibration and validation purposes.

The structure of the paper is as follows: in Section 2 the experimental measurements used for calibration and validation purposes are presented. In Section 3 the proposed model for electron transport description is showed. In Section 4 the calibration and validation results are presented and discussed. Some final remarks conclude the paper. The equations of the original model proposed by [9] has been reported in Appendix B for the sake of completeness.

2. Material and methods

The microalgae strain *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 multi-cultivator MC 1000-OD system (Photon Systems Instruments, Czech Republic) at a temperature of 21 °C and a light intensity of $100\,\mu E\,m^{-2}\,s^{-1}$ provided continuously by an array of white LEDs. The suspension culture was constantly mixed and aerated by bubbling air. Pre-cultures were grown at $100\,\mu E\,m^{-2}\,s^{-1}$ in glass bottles of 0.25 L under a continuous airflow, enriched with 5% CO_2. After reaching the exponential growth phase, the pre-culture was centrifuged and re-suspended in fresh medium to have a final concentration of $9 \cdot 10^6$ cells mL^{-1}, before introduction in the multi-cultivator. The culture analyzed was kept in exponential phase by dilution with fresh medium every 2–3 days.

Spectroscopic analyses were performed in vivo using a Joliot-type spectrophotometer (JTS-10, Biologic, France) as described in detail in [12,16]. The analyses performed are focused on PSI and in particular on P700, the primary electron donor present in the PSI reaction center. Oxidised PSI ($P700^+$) can be detected by a differential absorption signal at 705 nm that thus can be exploited to quantify the PSI oxidation kinetics. The experimental task was conducted by exposing samples containing $300 \cdot 10^6$ cells mL^{-1} to actinic lights of different intensities (from limiting to saturating actinic light): 80-150-320-940-2050 $\mu E\,m^{-2}\,s^{-1}$. The light was on for 15000 ms to reach a steady oxidation state of P700. At the end of each light treatment, the light was switched off, allowing for the P700 re-reduction. The maximal amount of photo-oxidizable P700 was quantified by the absorption signal obtained by exposing to saturating light ($2050\,\mu E\,m^{-2}\,s^{-1}$) through cells pretreated with 2,5-Dibrom-3-methyl-6-isopropyl-p-benzochinon (DBMIB), a compound that blocks plastoquinol oxidation by the cytochrome b6f complex therefore promoting full oxidation of PSI.

The measurement variable used for models calibration is the oxidised fraction of PSI centers ($x_{PSI,2}$), obtained as the ratio between the steady state absorbance signal reached with illumination at different actinic light intensities and maximal photo-oxidizable P700 obtained with DBMIB-poisoned cells.

Joliot-type spectrometer also allows to estimate the electron flows through the photosynthetic electron transport chain, which will be used for validation in this paper. In particular, the total electron flow (TEF), i.e. the sum of all the electron transport through PSI, obtained at different light treatment was estimated measuring the oxidised P700 re-reduction rates after each illumination in untreated cells. By multiplying this rate constant by oxidised fraction of PSI centers at a given light (obtained by comparison with poisoned cells treated with $2050\,\mu E\,m^{-2}\,s^{-1}$, i.e. the maximal level of P700 oxidation) it is possible to quantify the total electron flux through the photosystem [16]. The same procedure was repeated in samples treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), that blocks PSII activity, and allows assessing the contribution of the linear (PSII + PSI dependent) and cyclic electron pathways (only PSI-dependent) to the total electron flow.

3. Modelling the electron transport chain

Fig. 1 represents the electron transport chain scheme considered in this work. Two alternative models have also been considered and are described in detail in Appendix A. The PSII activity is described using the model by [9], which for the sake of completeness is reported and described in Appendix B. The electron flux from PSII is equal to the quantity B/τ_{PSII}, where B is a variable in [9] representing the fraction of closed reaction centers and τ_{PSII} is a parameter representing the turnover rate of PSII and is kept fixed to the value considered in [9] and included in Table B.5. Between the two photosystems a series of three different electron carriers is responsible of the transport of electrons from PSII to PSI, namely plastoquinone (PQ), cytochrome b6f complex (Cytb6f) and, for the case of *N. gaditana*, cytochrome c6 complex (Cytc6) instead of Plastocyanin. Downstream of PSI another electron carrier is present, the ferredoxin (Fd) [17]. The electron transport is conventionally split into two main electron fluxes: the linear electron flow (LEF) and the cyclic electron flow (CEF) (see [14]). Some authors also introduced the pseudo-linear cyclic electron flow, in order to describe the complex transient behaviour of the electron transport chain just after light activation after long dark periods [18]. However as several other photosynthetic organisms [19], *N. gaditana* genome is missing sequences encoding for FLV proteins, the major responsible of

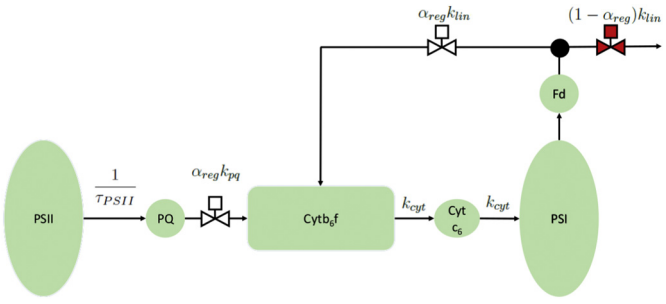

Fig. 1. *CEF regulation* model flowsheet. The arrows show the connections (and the related kinetic constants) between the protein complexes involved in the electron transport. The valves are colored in white or red to highlight their opposite operation: when the white valves close the red valve opens accordingly. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)