Algal Research 17 (2016) 21–30

Contents lists available at ScienceDirect

Algal Research

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

Chlorella fusca (Chlorophyta) grown in thin-layer cascades: Estimation of biomass productivity by *in-vivo* chlorophyll *a* fluorescence monitoring

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ARTICLE INFO

Article history:
Received 29 November 2015
Received in revised form 12 April 2016
Accepted 13 April 2016
Available online xxxx

Keywords:
Biomass productivity
Chlorella
Electron transport rate (ETR)
Microalgae
In vivo chlorophyll *a* fluorescence

ABSTRACT

The microalga *Chlorella fusca* showed high biomass productivity in thin-layer cascades (TLCs) with large light-exposed surface (high exposed surface to total volume ratio; S/V). Two trials – E1 and E2 – were conducted in Málaga (Southern Spain) in July and October, respectively using a TLC with an S/V ratio of 27 m^{-1}. The third trial E3 was carried out in the Czech Republic in July using another TLC with an S/V ratio of 120 m^{-1}. Chl *a* fluorescence measurements (*in situ* and off-line) were carried out to monitor the photosynthetic performance of these cultures. Simultaneous measurement of irradiance and effective quantum yield through the day allowed the determination of daily electron transport rate (ETR), which led to the estimation of biomass productivity. Calculated data of biomass productivity of microalgal mass cultures were well correlated with the measured values. The E3 culture achieved the highest productivity of the three trials −27 g DW m^{-2} d^{-1}. Extrapolating these results to southern Spain, with a longer cultivation period and higher daily dose of the solar energy, and considering a photosynthetic efficiency (PE) of 4.4% (achieved in E3), we obtained a potential productivity of 43 metric tons ha^{-1} $year^{-1}$ if the unit is operated for 8 months (from March to October). Realistically, in locations with favorable climate, TLCs can be considered a suitable system for biomass production with high PE as well as easy maintenance and low operation costs. On-line monitoring of *in situ* Chl *a* fluorescence provided data that revealed essential information about the photosynthetic performance of the culture. Here, we present a first attempt of estimating biomass productivity based on ETR measurements in microalgal mass cultures that correlate well with the measured values.

1. Introduction

Chlorophyll *a* (Chl) fluorescence has become a widespread technique to evaluate photosynthetic performance due to its non-invasiveness, sensitivity and a wide availability of commercial fluorometers that are easily usable *in situ* [1–3]. *In vivo* Chl *a* fluorescence reflects the utilization of light energy, *i.e.* its distribution between photochemical and non-photochemical processes. Several variables can be estimated using a pulse amplitude modulated (PAM) technique: the effective quantum yield ($\Delta F/F_m'$), used as an indicator of acclimation of photosystem II (*PSII*) as it depends on the redox state of the reaction centers; the maximal quantum yield (F_v/F_m'), which is an estimate of the maximal photochemical *PSII* efficiency and is used as an indicator of the physiological state of the culture, and the electron transport rate (ETR) through *PSII*, used as a proxy of the photosynthetic capacity and productivity [4,5]. For decades, chlorophyll *a* fluorescence has been extensively applied in plants [6–9], microalgal photosynthesis [10–13] and microalgal ecophysiology [2,14–16].

In the last decade, microalgal biotechnology has received great attention as it can potentially reach high biomass yield [17] to extract valuable products [18–22]. Most of currently used cultivation systems are outdoor units. These are exposed to a variation of environmental conditions (*i.e.* irradiance, temperature, pH, gas exchange, hydrodynamics and nutrition) that directly influence biomass productivity. Thus, it is desirable to obtain immediate response to unfavorable conditions that may decrease culture biomass productivity. PAM fluorometry has been successfully used on-line to monitor the photosynthetic performance of mass cultures and give rapid evidence of stress affecting growth [4,23–25].

Primary photosynthetic productivity has been traditionally measured in laboratory by gas exchange techniques, *i.e.* oxygen evolution and CO_2 assimilation. However, they present several these techniques present a major drawback: long time is required to complete the measurements. Due to the advantages of *in situ* Chl *a* fluorescence measurements, PAM-derived ETR measurements have been used as an estimate

Abbreviations: Chl, chlorophyll; DW, dry weight; E1, E2, E3, various cultivation trials; NPQ, non-photochemical quenching; *PSII*, photosystem II; TLC-ES, TLC-CZ, thin layer cascades located in Málaga (Spain) and Třeboň (Czech Republic), respectively; Y(*PSII*), actual photochemical yield of *PSII*; PE, photosynthetic efficiency.

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http://dx.doi.org/10.1016/j.algal.2016.04.010
2211-9264/

of biomass. Good correlations between ETR and C-fixation or oxygen evolution have been reported in laboratory measurements [4,5,23,26–28] although most of these studies focused on natural aquatic ecosystems, as documented by Suggett et al. [29]. A few studies conducted in outdoor mass cultures recorded $\Delta F/F_m'$ [24,25,30] and only some of them measured simultaneously PAR irradiance inside the culture to obtain relative ETR values ($rETR = E_{PAR} \times \Delta F'/F_m'$) [25,26,31].

In the present study, we used *in vivo* Chl *a* fluorescence to monitor the photosynthetic performance of outdoor cultures of the microalga *Chlorella fusca*. In microalgal biotechnology the measurement of biomass productivity is usually carried out through the timely assay of dry weight. In this study, we estimate biomass productivity from the daily integrated electron transport rate by using *in situ* measurements of *in vivo* chlorophyll *a* fluorescence (measured continuously at 5-min intervals). This manuscript represents a first attempt to predict biomass yield using *in vivo* Chl *a* fluorescence in mass cultures and it includes useful information to identify critical points in algal growth during the day to get a good optimization of the outdoor cultures. This non-intrusive technique provides information on the physiological status of microalgal cultures, which is not possible to obtain by oxygen evolution measurements.

2. Material and methods

2.1. Species and culture conditions

Laboratory cultures of the microalga *C. fusca* (strain BEA1005B, from the Spanish Collection of Algae — BEA) were grown in Bold's Basal Medium fortified with a 3-fold nitrate content and the addition of vitamins (3*N*-BBM-V) [32,33] under irradiance of 150 μmol photons m^{-2} s^{-1} at 25 °C (12 h light:12 h dark). Then, the laboratory culture was transferred to an outdoor thin-layer cascade (TLC) and diluted to biomass density of 0.1–0.2 g DW L^{-1}.

Table 1 summarizes the growth conditions of outdoor trials, the first two, E1 and E2, conducted in Málaga (Spain) in July and October 2012, respectively using the TLC-ES unit. The third trial, E3, was carried out in Třeboň (Czech Republic) in July 2013 using the TLC-CZ unit. Despite their location, both TLC systems differ in dimension, and thus, also in the time the culture spend in the exposed area. In the TLC-ES the culture flows along an exposed surface of 4 m^2 (cascade and basin), which, according to the hydrodynamics data reported previously [31], has a retention time of ~26%. The rest of the time the culture is in the tank (~66%) or in the dark section (pump and pipes, ~5%). In the TLC-CZ the microalgal suspension flows along a declined surface of 24 m^2 and a volume of ~170–225 L [25]. These differences are pointed out in the surface to volume ratio of the systems (shown in Table 1) –27 m^{-1} in TLC-ES and 120 m^{-1} in TLC-CZ. The depth of the culture layer on the cascade was 6–8 mm in both systems.

Table 1
Set-up and culture conditions during three outdoor trials E1, E2 and E3 with *Chlorella fusca*.

	E1	E2	E3
Location	Málaga, Spain 36°43′ N 4°25′ W	Málaga, Spain 36°43′ N 4°25′ W	Třeboň, Czech Republic 49°00′ N 14°46′ E
Date	July 2012	October 2012	July 2013
TLC system	TLC-ES	TLC-ES	TLC-CZ
S/V ratio (m^{-1})	27	27	120
Daily cycle (light:dark)	14:10	12:12	13:11
Temperature range (°C) (min/max)	20/36	15/27	14/32
Average PAR daily dose (mmol m^{-2})	51,203	29,093	38,711
pH	7.4–7.8	7.4–7.8	7.4–7.8
Initial biomass density (g L^{-1})	0.1	0.15	0.42
Final biomass density (g L^{-1})	1.22	0.92	3.65

2.2. Analytical procedures

Dry weight and content of total internal carbon analysis are presented in another manuscript (Jerez et al., in preparation) and were used in the present study for calculations explained in the following sections. Briefly, dry weight measurements were carried out by centrifugation of 2-mL biomass and dried using pre-weighted GF/C filters and total internal carbon content was determined from dried biomass using an elemental analyzer (LECO CHNS 932).

Biomass productivity (hereafter measured biomass productivity, mBP) was calculated based on dry weight increase within 24 h and expressed as [g DW L^{-1} d^{-1}].

The optical density was measured (400–700 nm) using UV–VIS spectrophotometer (UVmini-1240, Shimadzu, Japan) before and after pigment extraction with dimethyl-formamide (DMF). The average absorption coefficient (λ: 400–700 nm) was calculated (a, m^{-1}) according to Arbones et al. [34]. In addition, light absorption was also assessed by measuring the absorptance according to Jerez et al. [35]. We used the equation $A = 1 - E_p / E_m$, where E_p/E_m is the transmittance (T), E_p is the transmitted irradiance by the microalgal culture and E_m is the transmitted irradiance by the culture medium, both measured with a cosine corrected PAR sensor LI-COR 192SA (LI-COR Company, Nebraska, USA). Both the absorption coefficient (a) and the absorptance (A) were measured in triplicate 4 times a day at 8:00, 12:00, 16:00 and 20:00 h.

2.3. In vivo Chl a fluorescence measurements

Measurements of *in situ* Chl *a* fluorescence were recorded at 5-min intervals during the day using Junior-PAM fluorometer (Walz GmbH, Effeltrich, Germany). A plastic fiber optic (100 cm, 1.5 mm diameter) and a spherical PAR sensor (US-SQS, Walz GmbH, Effeltrich, Germany) were submerged directly into the microalgal culture to record Chl *a* fluorescence and PAR irradiance simultaneously (see details of the measuring system in Fig. 1). As described previously [25,31], in TLC-ES sensors were placed at a depth of 6 mm (in the basin) and at 3-mm deep in the middle of the culture layer in the cascade of the TLC-CZ. Blue light-emitting diodes (LED, 460 nm) were used to apply the saturating pulses, measuring and actinic lights controlled by PC *via* USB interface. WinControl-3 software was used for data acquisition and recording.

Using the measurement setup detailed above, the incident photosynthetically active irradiance (E_{PAR}) and the effective quantum yield, $\Delta F/F_m'$, were measured every 5 min during the light period, where: $\Delta F = F_m' - F_t$; F_m' is the maximum fluorescence yield of an illuminated sample and F_t is the instantaneous fluorescence of illuminated algae measured before the application of the saturation pulse. The relative electron transport rate through *PSII* (rETR; r.u.) was determined as follows:

$$rETR = \Delta F/F_m' \times E_{PAR}. \quad (1)$$

Although rETR is frequently used to estimate biomass productivity, it is a relative value and should be cautiously considered since photobiochemical responses depend on the absorbed light and not on the incident irradiance [36]. Thus, we recalculated rETR to absolute ETR values taking into account light absorption by *PSII*, which was estimated by measurements of (1) absorptance (A, dimensionless), or (2) absorption coefficient (a, m^{-1}). The A value, which is the fraction of light absorbed by the culture, was measured by a method used for macroalgae [37–40] and modified to microalgae cultures [4,35]. In this method, the absorptance is calculated as $A = 1 - E_p / E_m$, where E_p / E_m is the transmittance (T); E_p is the transmitted irradiance by the