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LIGHT INTENSITIES MAXIMIZING PHOTOSYNTHESIS AND KINETICS OF PHOTOCHEMICAL STEPS IN *Graesiella emersonii* UNDER DIFFERENT CULTIVATION STRATEGIES

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Abstract

The aim of this paper is studying light intensity values which maximize the photosynthesis in microalgal cultures by means of models of the photochemical process and by changing cultures strategies.
The photosynthetic performances of *Graesiella emersonii* under batch, fed-batch, semi-continuous cultivation modes and with only air sparged or CO_2 added to air were quantified by means of gas exchange measure and pulse amplitude modulated fluorimetry (PAM); kinetics of the photochemical processes was determined processing data from PAM and using the well-known Eilers and Peeters model. Both PAM, via the kinetic model, and gas exchange techniques allowed to identify similar light intensities maximizing the photosynthesis rate at least when CO_2 was added to air.
When CO_2 wasn't added some discrepancies appeared between the two methods used. These discrepancies seem to suggest that, in suffering conditions and in presence of some cumulative effects, the kinetic model used could be less accurate and perhaps need some adjustments.

Key words: Graesiella emersonii microalgae, photobioreactor, photosynthesis, pulse amplitude modulated fluorimetry

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1. Introduction

Light is one of the most critical factors of intensive microalgal cultivation (Olivieri et al., 2014). Indeed, light should be provided at the appropriate intensity and wavelength, since both too low or excessive light intensities can be growth-limiting (e.g. Carvalho et al., 2010; Chen et al., 2011).

Several kinetic models have been developed to forecast and describe this behavior in microalgae. Peeters and Eilers (1978) and, more extensively, Eilers and Peeters (1988) proposed a simple, linear model (E P model), which was also modified by considering e.g. photoadaptation, photoinhibition and flashing light effect (Bannister, 1979; Bernardi et al., 2014; Camacho-Rubio et al., 2003; Garcia-Camacho et al., 2012; Han et al., 2000; Nikolaou et al., 2015).

They assumed three possible states for the photosynthetic reaction centers (open or resting state (1) in the dark, closed or activated state (2) in the presence of light, damaged or photoinhibited state (3) due to excess of light) and four kinds of transitions among the states characterized by four kinetic parameters (α, β, γ, δ). Now let x_1, x_2, x_3 denote the fractions of the reaction centers respectively in the states (1), (2), (3) and I the light intensity (Fig. 1).

A first kind of transition happens when the open state is activated by the photon capture and goes

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to the closed state. The photon capture rate r_{PC} is assumed to be proportional to I, x_1 and α. A second form of the transition happens when the closed state returns to the open state thanks to the photochemical quenching. The rate of photochemical quenching r_{PQ} is assumed to be proportional to γ and x_2. A third kind of transition happens when the closed state turns into photoinhibited state. The rate of the photoinhibition r_{PI} is assumed to be proportional to I, x_2 and β. A last type of the transition happens when the photoinhibited state recovers the open state by repairing process. The rate of the repairing process r_{REP} is assumed to be proportional to δ and x_3.

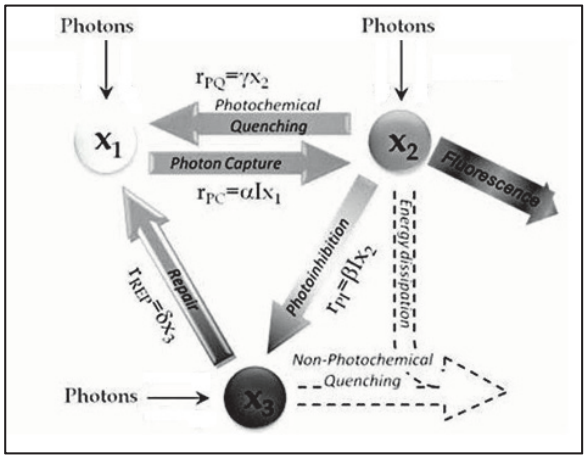

Fig. 1. Schematic representation of Eilers and Peeters' kinetic model (from Gargano et al., 2015, modified)

Then the time evolution of x_1, x_2, x_3, is described by the system of ordinary differential equations (Eq. 1) with initial conditions $x_1(0) = 1$, $x_2(0) = 0$, $x_3(0) = 0$.

$$\begin{aligned}
\frac{dx_1}{dt} &= aIx_1 + \gamma x_2 + \partial x_3 \\
\frac{dx_2}{dt} &= aIx_1 + \gamma x_2 + \beta Ix_2 \\
\frac{dx_3}{dt} &= \beta Ix_2 - \delta x_3 \\
x_1 x_2 + x_3 &= 1
\end{aligned} \tag{1}$$

The model allows to calculate the light intensity (I_{opt}) maximizing the photochemical process by avoiding the inhibited status x_3. It is given by Eq. (2):

$$I_{opt} = \sqrt{\frac{\delta\gamma}{\alpha\beta}} I_{opt} = \sqrt{\frac{\delta\gamma}{\alpha\beta}}, -, -, -..I_{opt} = \sqrt{\frac{\delta\gamma}{\alpha\beta}} \tag{2}$$

Recently, Gargano et al. (2015) proposed a procedure to assess the kinetic parameters by pulse amplitude modulation fluorimetry. The procedure was applied on several microalgal strains and different photobioreactor designs, confirming that the performance of photochemical processes reflects the physiological state and growth of each algal strain, and plays a key role in the selection of the most suitable cultivation regime to be adopted in mass cultures, as stressed by Malapasqua et al. (2014).

Recently, we have showed that the alga is a good candidate both for biodiesel production and the phycoremediation of hazardous phenolic pollutants (Carbone et al. 2017a, Carbone et al. 2017b, Carbone et al. 2018). A first aim of the present paper is to investigate the effect of the cultivation modes (batch, fed-batch and semi-continuous) and CO_2 addition in the gas phase on *G. emersonii* and on the dynamic of photochemical steps involved in the E P model and to asses the optimal values of light intensity I_{opt} maximizing the photosynthesis rate.

A second purpose of this paper is to evaluate the efficiency of the photochemical process by using the gas exchange analysis again in different cultivation strategies. More precisely for the gas exchange analysis, Henley (1993) proposed an equation (Eq. 3) linking experimental data i.e. the photosynthesis rate (P), photon flow rate (PFR), the respiration rate in darkness (R_d) to the photosynthesis rate at saturating PFR (P_{max}) and to ascending initial slope (α_I) of the curve *P-PFR*:

$$P = P_{\max}\left[\frac{\alpha_i PFR}{P_{\max} + \alpha_1 PFR}\right] + R_d \tag{3}$$

By this equation, it is possible to estimate the value of P_{max} and (α_I) as such ones that the Eq. (2) better fits the experimental curve P-PFR. The onset of saturation light (E_k) is estimated by Eq. (4) (Mercado et al. 2004). Then we evaluate E_k in all described cultivation strategies.

$$E_k = \frac{P_{\max}}{\alpha_1} E_k = \frac{P_{\max}}{\alpha_1} E_k = \frac{P_{\max}}{\alpha_1} \tag{4}$$

The results obtained by integrating fluorometric measurements with the gas exchange analysis will be used to evaluate the efficiency of photochemical processes under different strategies of cultivation, acquiring useful data to improve the productivity of *G. emersonii* mass cultures. As a consequence of our results, we also discuss some aspects of E and P model adequacy.

2. Materials and methods

2.1. Microalgal strains and medium

The strain 211/11m *Graesiella emersonii* from CCAP collection was selected for the experiments. This strain, originally designed *Chlorella emersonii* by Shihira and Kraus, was subsequently transferred to *Scenedesmus,* as *S. vacuolatus,* by Kessler et al. 1997; it has been renamed in 2017 as *Graesiella emersonii* by Nozaki, Katagiri, Nakagawa, Aizawa and Watanabe after a taxonomic review (https://www.ccap.ac.uk).

Modified Bold Basal Medium (BBM) as reported by Olivieri et al. (2013) was used. The

medium was autoclaved for 20 minutes at 120°C. The final pH of the medium was close to 7.

2.2. Photobioreactor design and operating conditions

Microalgae were grown in vertical cylindrical bubbles column photobioreactors. Design details can be found elsewhere (Olivieri et al., 2012). The irradiance level was set at 250 μE/(m^2 s^1). Preliminary measurements of photosynthesis rate by gas exchange method showed that this value can provide no photolimtation behavior in the culture. Moreover some control experiments were done at a very low level of light intensity 40 μE/(m^2 s^1) under 2% CO_2 and air sparged. Biomass yield were similar (almost 5 g/L of biomass in fed-batch mode under 2% CO_2 and 2 g/L under air sparged) but it took a longer time to achieve it (30 days). To avoid high surface cell concentration, a regular bubbling was provoked in the photobioreactor and, consequently, a mixing was determined in the culture.

The CO_2 concentration in the gas stream fed to the photobioreactors was increased from only air sparged to 2% (2% CO_2). Tests were carried out under batch conditions (B) for about one week. The shift to the fed-batch phase (F-B) took place after ten days when the nitrogen concentration in the medium achieved a value lower than 10 mg L^{-1}. Under fed-batch mode, ten times concentrated medium was added to the cultures twice a week in order to avoid the nutrient starvation and to allow the continuous cell growth. The final volume of the culture was kept constant because of the water withdraw due to the samples and to water stripping by gas bubbles. The shift to the semi continuous phase (SM) took place after nineteen days. Under the SM, 30% of culture was replaced with three times concentrated fresh medium every seven days in order to achieve a dilution rate equal to 0.043 d^{-1} and to replace the initial concentration of the nutrient. The reactors where operating under the SM for about three weeks until the biomass steady state was achieved. The biomass and medium collected during the forty days of cultivation under the B, F-B, SM phases were characterized. Microalgal cultures were carried out in duplicate for each set of operating conditions.

2.3. Medium characterization

pH: the pH was measured with a pH meter (benchtop pH meter Mettler Toledo).

Nitrate: the analysis of nitrate concentration in the medium was made using a nitrate electrode (Metter-Toledo).

2.4. Biomass characterization

Biomass concentration: the biomass concentration was estimated by measuring the optical density at 600 nm with a spectrophotometer (Specord 50 – Analytic Jena) and then dry weight was measured by analytic balance.

Chlorophyll *a (Chl* a*)* content: the *in vivo* chlorophyll *a* content was measured with a fluorometer (AquaFluorTM; Handheld Fluorometer/Turbidimeter; Turner Designs), and the count of cells was carried out by a Burker bloodcounting chamber.

2.5. Gas exchange

Oxygen evolution in the samples was measured in an oxygraphy (Oxygraph Hansatech) equipped with a thermostatic control set at 24°C and with S1 Clark-type oxygen electrodes. Different light intensities (PFR) were supplied ranging from 43 to 600 μE/(m^2 s^1). The oxygen control unit allowed to observe the oxygen consumption during the O_2 uptake under the dark phase R_d and the oxygen production under the light phase P. The biomass concentration was always reported to 0.12 g/L for all gas exchange measurements in order to avoid the light limitation. The values of oxygen measured in dark and light condition were before normalized with cell number and after for Chl *a* content in the culture. So, P and R_d were expressed as μmol_{O2} μg_{Chla}^{-1} s^{-1} (per cell).

Gas exchange measurements were carried out in triplicate for each set of operating conditions. Three minutes of dark for R_d measurements and three minutes of light for P measurement were alternated.

2.6. Fluorescence measurements

Chlorophyll fluorescence emissions were determined at room temperature by a pulse amplitude modulated fluorimetry (Hansatech Fluorescence Monitoring System). Microalgae, before the measurements started, were adapted to darkness for 30 minutes; during all experiments, the sample was continuously stirred. The analysis was based on two different measurement techniques, chlorophyll response to light pulse (LP) and saturating light pulse under modulated light (ML) conditions and chlorophyll response to saturating light pulse (SP) under actinic light conditions (AL) for three minutes (Carbone et al., 2017b).

During the experiment, fluorescence measurements were carried out at different cell densities in the exanimated density range. The results were not relevantly affected by the variations of this parameter (data not shown). Every time that the biomass and chlorophyll data were obtained, fluorescence measures were together made. Fluorescence measurements were carried out in triplicate for each set of operating conditions.

2.7. Assessment of kinetic parameters

The kinetic parameters were assessed by processing fluorescence data with a code developed in Matlab programming language. Parameters α and γ were estimated through the elaboration of fluorescence data achieved with light pulse (LP) under modulated light condition (ML).

Indeed, in these conditions, pulse actives the open state and we can assume that only transitions of the population of reaction centers from (1) to (2) or (2) to (1) happen. Then we calculated time evolution of x_2 and its maximum value X_2 by neglecting the term x_3 in the differential system of figure 1, using x_1=1, x_2=0 as initial values for assigned values of α and γ. On the other side we measured the basal fluorescence F_0, the maximum fluorescence value F_M and the instantaneous fluorescence F. Eventually, we chose the values of α and γ such that the ratio x_2/X_2 better fitted the ratio $(F-F_0)/(F_M-F_0)$. Parameter β was estimated through the elaboration of fluorescence data realized with saturating pulse (SP) under ML condition. During this pulse, it is possible to neglect the transition from state (3) to state (1) and so the term δx_3 in the differential system.

By using the values of α and γ previously obtained, we calculated time evolution of x_2 and its maximum value X_2 for assigned β and initial values $x_1=1$ $x_2=0$ $x_3=0$. We measured the instantaneous fluorescence F and maximum fluorescence value F_M. Again, we chose the value of β such that the ratio x_2/X_2 better fitted the ratio $(F-F_0)/(F_M-F_0)$. Parameter δ was estimated through the elaboration of fluorescence data realized during saturating pulse (SP) under actinic light (AL) conditions. Indeed, fluorescence in this condition is due to the transition among the three states, and under actinic light, it is possible to assume a steady state for the three states.

Then, firstly, we calculated the values x_1, x_2, x_3 in this steady state for an assigned value of δ by using the differential system, and previously obtained values for α, β, γ; secondly, we calculated the time evolution of x_3 assuming as initial values of x_1, x_2, x_3 the values of the steady state. We measured the maximum fluorescence value F_M, and the basal fluorescence after the saturating light pulse F' at different values of actinic light. Again, we chose the values of δ such that the value x_3 better fitted the ratio F'/F_M. We refer to Gargano et al. (2015) for more details.

The α and γ kinetic parameters were examined on biomass in dark adapted condition using pulse characterized by a light intensity ranging from 500 to 3000 μE/(m^2 s^1) and a pulse amplitude from 0.6 to 2 s. The β constant was estimated on biomass in dark adapted condition using saturating pulse characterized by light intensity ranged from 3000 μE/(m^{-2} s^1) to 4000 μE/(m^2 s^1) and pulse amplitude from 0.8 to 2 s. The δ term was calculated on biomass in light adapted condition; light condition ranged from 40 to 1350 μE/(m^2 s^1) while saturating pulse was set at 3000 μE/(m^2 s^1) for 0.6 s. We observe explicitly that parameters $\alpha, \beta, \gamma, \delta$, in E P model, are independent on the light intensity. So we can use high intensity light and light pulse to evaluate them, too.

3. Results and discussion

3.1. Biomass growth

Fig. 2 shows data for cultures of *G. emersonii* carried out in vertical bubbles column photobioreactor feeding with 2% CO_2.

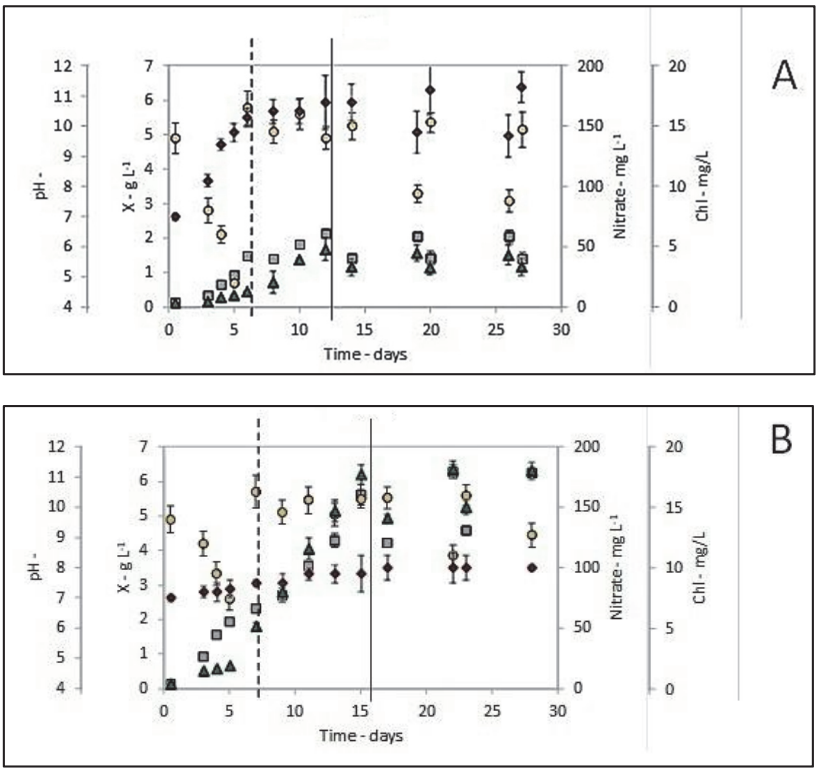

Fig. 2. Cultures of *G. emersonii* at different operating conditions: (A) represents the culture with only air sparged while (B) represents the culture with CO_2 added. Dashed lines mark the beginning of fed-batch state. Continuous lines mark the beginning of semicontinuous mode. Biomass concentration [X] was indicated by squares. Chorophyll *a* concentration was indicated by triagles. pH was indicated by rhombus. Nitrate concentration was indicated by circles

The biomass concentration increased up to about 2.0 g/L in the B phase and then achieved a maximum value of about 5.5 g/L at the end of the F-B phase; it, at steady state in the SM phase, remained fairly constant. The Chl *a* content was about 0.5 μg/mL at the beginning of the B phase and was around 2 μg/mL at the end of this phase; it increased with the time in the cultures to about 18 μg/ml at the end of F-B phase. It, at steady state in SM phase slightly declined to around 16 μg/ml.

A similar trend was obtained for *G. emersonii* cultures grown with only air. The biomass concentration at the end of B phase resulted be 1.44 g/L while the Chl *a* content was 1.3 μg/mL. At the end of F-B phase, the biomass concentration achieved 2.16 g/L whereas Chl *a* content was 4.8 μg/mL. At the steady-state in SM phase, the biomass concentration was 1.6 g/L while the Chl *a* content reached a value of 3.8 μg/mL. In both type of experiment in B phase, the Chl a didn't increase a lot its values compared to the biomass because already from the first measurements this pigment suffered from conditions of N-starvation typical of batch phase (Evans, 1989).

The N-starvation was prevented by detecting the level of nitrate. In particular this check was done before feeding further medium to the culture in case of FB and SM and at the end of the growth for B. So, nitrate concentration was never lower than 150mg L^{-1} for longer than one day.

3.2. Photosynthetic behavior by fluorometric analysis

Table 1 reports the values of kinetic parameters of the photochemical processes assessed for *G. emersonii* cultures carried out under B, F-B and SM operating condition in 2% CO_2. During the experiment pH slightly change: it was around 7.2 in B phase, 7.8 in F-B phase and 8 in SM phase. In this operating condition, the values of ''capture'' coefficient α and ''quenching'' coefficient γ were consistent within the limits of experimental errors. The values of ''photoinhibition'' coefficient β and "repairing" coefficient δ were consistent within the limits of experimental errors in B and SM phases and not consistent (and relatively higher) in F-B phase, but the orders of magnitude didn't change. So, passages from state (1) to state (2) and vice versa appear to be independent on operating condition; passage from state (2) to state (3) and from state (3) to state (1) slightly depend on operating condition and seem to be more efficient when the wealth-state of the culture is better as in F-B phase. Then the changes in the values of β and δ suggest that the Peeters and Eilers model should slightly more accurately take account on the algae wealth-state at least when this state is very good.

In this regard, we can observe that also the fluorescence is influenced by the physiological status of the microalgae (Serôdio et al. 2009). Table 2 reports the assessed values of kinetic parameters for the *G. emersonii* cultures grown in only air condition.

During this experiment, pH strongly changed: it was around 7 at the beginning, 10,3 at the end of B phase, 10,8 in F-B phase 11,2 in SM phase. This was a clear symptom of microalgae culture suffering state.

The values of γ were almost consistent within the limits of experimental errors in all operating conditions. The values of α were consistent only within two times the limits of experimental errors. The maximum values of α and γ were assumed in F-B phase. The values of δ were consistent within the limits of experimental error in B and F-B phases; the value in SM phase was lower, but the order of magnitude didn't change. The values of β were consistent within the limits of experimental errors in F-B and SM phases; the maximum values was assumed in B-phase and order of magnitude changed.

In this operating condition, the microalgae wealth-state was very poor, as shown in Fig. 2A by pH values (see also Miller et al.1988) and confirmed by lower ratios of chlorophyll a to biomass, starting from N starvation at the end of B phase. So, an accumulation effect was likely produced and some metabolic adaptations seemed to be determined. Then E P model seems to be less accurate. More precisely, comparing the results obtained in the two operating conditions, we can observe that the changes in order of magnitude occurred for δ (in every operating mode) and for β (in B-phase). So, these two parameters (and in particular the "repairing" parameter δ) are more sensitive.

Table 1. Kinetic parameters of the photochemical processes assessed for *G. emersonii* cultures under B, F-B and SM conditions in 2% CO_2 mode

	B	***F-B***	***SM***
‹ (m^2/μE)	$1.55\cdot10^{-3}\pm5.7\cdot10^{-4}$	$1.13\cdot10^{-3}\pm8.47\cdot10^{-4}$	$9.51\cdot10^{-4}\pm1.81\cdot10^{-4}$
β (m^2/μE)	$2.78\cdot10^{-4}\pm5.4\cdot10^{-5}$	$5.56\cdot10^{-4}\pm6.67\cdot10^{-5}$	$2.58\cdot10^{-4}\pm5.80\cdot10^{-5}$
© (s^{-1})	5.3 ±0.90	3.99±0.54	4.18±0.19
δ (s^{-1})	$1.02\cdot10^{-2}\pm3.2\cdot10^{-3}$	$2.39\cdot10^{-2}\pm1.7\cdot10^{-3}$	$8.77\cdot10^{-3}\pm1.90\cdot10^{-3}$

Table 2. Kinetic parameters of the photochemical processes assessed for *G. emersonii* cultures under B, F-B and SM modes in only air condition

	B	***F-B***	***SM***
‹ (m^2/μE)	$2.34\cdot10^{-3}\pm2.54\cdot10^{-4}$	$4.02\cdot10^{-3}\pm6.87\cdot10^{-4}$	$1.27\cdot10^{-3}\pm2.99\cdot10^{-4}$
β (m^2/μE)	$1.85\cdot10^{-3}\pm1.97\cdot10^{-4}$	$7.87\cdot10^{-5}\pm2.67\cdot10^{-5}$	$1.17\cdot10^{-4}\pm1.98\cdot10^{-5}$
© (s^{-1})	5.99±0.41	6.83±0.39	6.75±0.26
δ (s^{-1})	$1.73\cdot10^{-3}\pm2.47\cdot10^{-4}$	$1.92\cdot10^{-3}\pm3.65\cdot10^{-4}$	$8.31\cdot10^{-4}\pm1.11\cdot10^{-4}$

By using Eq. (2), it was possible to calculate I_{opt}. The results are reported in Table 3. The value of light intensity I_{opt} that should avoid the formation of inhibited state resulted quite constant at varying the operating modalities for the same cultures conditions, but higher in the cultures with 2% CO_2. It is also interesting to note that this value seems to be linked to the average level of irradiance within the system (Olivieri et al., 2014, 2015), and that is determined not only by the wall irradiance, but also by the light path and cell density.

Table 3. Values of the light intensity I_{opt} maximizing the photochemical process

		B	***FB***	***SM***
2% CO_2	**I_{opt} (µE/(m^2 s^1))**	354	390	386
Only air	**I_{opt} (µE/(m^2 s^1))**	154	204	194

3.3. Photosynthetic behavior by gas exchange

Typical photosynthesis/respiration (P/I) curves obtained for both operating conditions and different operating mode are reported in Fig. 3 (after normalization). Data were used to estimate the value of photosynthesis characteristic parameter, the initial slope of the P/I curve (α_I) and the saturatig light irradiance value (P_{max}) according to Eq. (3). The best fitting values for α_I and P_{max} are reported in Table 4. The onset of light saturation E_k, calculated by using Eq. 4, is also reported. The highest value of P_{max} was obtained in SM operating mode, the lowest value in B phase, when the biomass concentration was lowest.

The initial slope of the P/I curves α_I slightly changed with the operating strategies and modalities. Only the value at SM modality with only air was significantly higher. The values E_k were higher in the cultures grown with 2% CO_2 and didn't much change with operation modes.

It has to be highlighted that the increase of biomass from B to F-B and SM can in principle generally results in some cumulative effect. This effect could be linked to some limitation of the supply of light, nutrient or CO_2. However, the level of irradiance as indicated guarantees no light limitation.

Table 4. Parameter of photosynthesis (P_{max} and α_I) and onset of light saturation (E_k) for all operating strategies

		P_{max} $[\mu mol_{O2}/(\mu g_{Chl\ a}\ s^1)]$	α_I $[\mu mol_{O2}/(\mu g_{Chl\ a}\ s^1/(\mu E/m^2\ s))]$	E_k $[\mu E/m^2\ s^1]$
B	2% CO_2	$2.9\cdot10^{-1}\pm5.8\cdot10^{-3}$	$9\cdot10^{-4}\pm1.3\cdot10^{-4}$	322
	Only air	$2.7\cdot10^{-1}\pm1.1\cdot10^{-2}$	$1.1\cdot10^{-3}\pm7.7\cdot10^{-4}$	245
F-B	2%CO_2	$4.0\cdot10^{-1}\pm1.3\cdot10^{-2}$	$1.2\cdot10^{-3}\pm1.6\cdot10^{-4}$	333
	Only air	$3.0\cdot10^{-1}\pm8\cdot10^{-3}$	$1.2\cdot10^{-3}\pm1.9\cdot10^{-4}$	250
SM	2% CO_2	$4.8\cdot10^{-1}\pm8{,}1\cdot10^{-3}$	$1.2\cdot10^{-3}\pm2.1\cdot10^{-4}$	400
	Only air	$4.6\cdot10^{-1}\pm9.6\cdot10^{-3}$	$1.8\cdot10^{-3}\pm2.8\cdot10^{-4}$	255

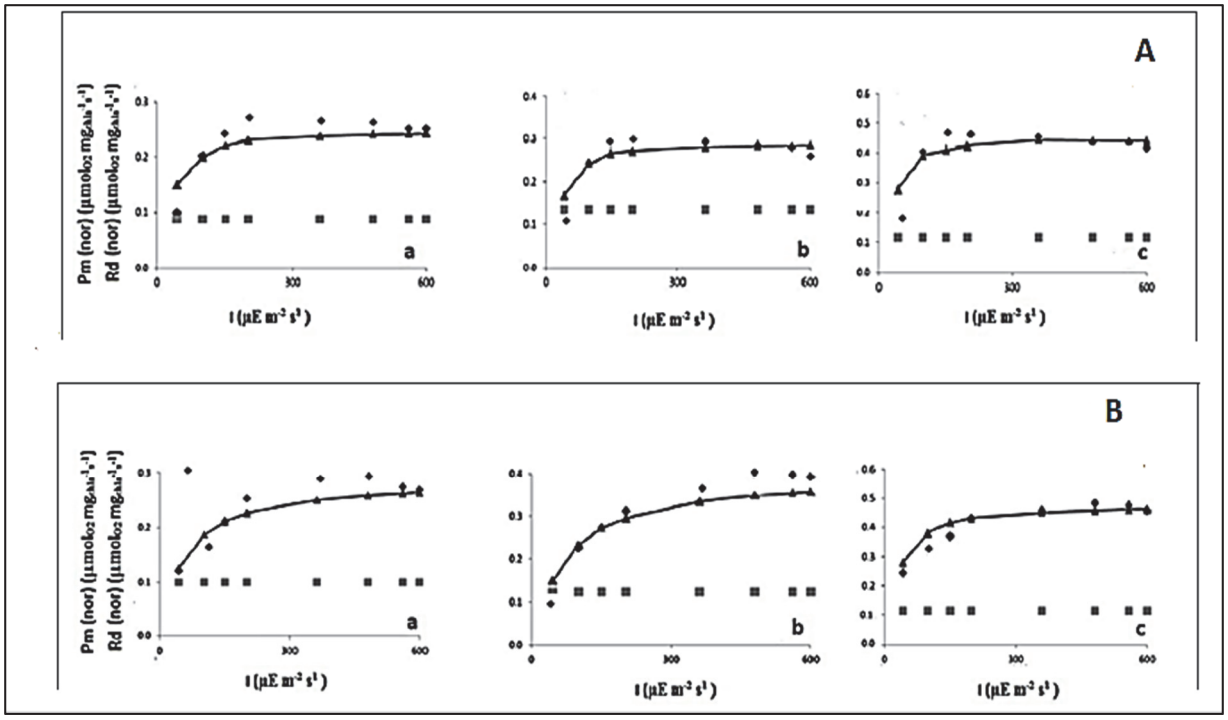

Fig. 3. Photosynthesis and respiration curve (P/I curve) of *G. emersonii* cultures under different operating strategies: (A) represents the culture with only air sparged while (B) represents the culture with CO_2 added. Squares show the normalized respiration in the dark, rhombus the normalized photosynthesis activity, triangles the modeling results. (a) represents Culture in B phase, (b) represents culture in F-B phase and (c) rapresents culture in SM phase.

The intrinsic nature of the operations (repeated supply of liquid nutrient) makes also unlikely a potential limitation by liquid nutrients except in B mode. The last possibility of limitation relies in the supply of CO_2. As observed, the culture grown under air only supply in the gas phase seems to be in a suffering state not as much by lower value of biomass yield, as by increasing value of pH and by a lower ratio of chlorophyll a to biomass, starting from B mode. This suffering state could also explain the lower value of the light intensity optimizing the photochemical quenching was lower.

4. Conclusions

The light intensity I_{opt} that maximizes the photochemical process by avoiding inhibited state resulted quite constant varying the operating modes, but it resulted higher in the cultures with 2% CO_2.

Analogously, the values of the onset of light saturation E_k varied with the operating conditions but not with the operating modes. In particular, E_k was higher in the cultures grown with 2% CO_2 and lower in the cultures with only air.

The values obtained for I_{opt} and E_k were quite similar in presence of CO_2, despite the different principle behind the PAM, used to evaluate I_{opt}, and the gas exchange analysis, used to evaluate E_k (*e.g.* the first one is mainly dependent by the wall irradiance of the light pulse, the second one is a volumetric measurement performed by the oxygen sensors located at the bottom of the sample), and the different adopted models (the first one is more theoretical, the second one is essentially empirical).

This correspondence between I_{opt} and E_k exactly identifies specific light intensities that maximize photochemical process avoiding photoinhibition. So, we can obtain a useful data to improve *G. emersonii* mass culture productivity.

Moreover, the repairing process from photoinhibition appeared to be much faster in cultures grown with 2% CO_2 than in cultures grown with only air. This suggests that the microalgae wealth state stability should be considered implicit in E P model and, in particular, in deduction of equation (1). It could be interesting to evaluate the necessary modifications when microalgae wealth state changes by monitoring "repairing" parameter δ as function of *e.g.* the values of pH.

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