Quantizing photosynthetic performance of phytoplankton using photosynthesis-irradiance response models

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Abstract

Background: Clarifying the relationship between photosynthesis and irradiance and accurately quantizing photosynthetic performance are of importance to calculate the productivity of phytoplankton, whether in aquatic ecosystems modelling or obtaining more economical production.

Results: The photosynthetic performance of seven phytoplankton species was
characterized by four typical photosynthesis-irradiance (P-I) response models. However, the differences were found between the returned values to photosynthetic characteristics by different P-I models. The saturation irradiance ($I_{sat}$) was distinctly underestimated by model 1, and the maximum net photosynthetic rate ($P_{nmax}$) was quite distinct from its measured values, due to the asymptotic function of the model. Models 2 and 3 lost some foundation to photosynthetic mechanisms, that the returned $I_{sat}$ showed significant differences with the measured data. Model 4 for higher plants could reproduce the irradiance response trends of photosynthesis well for all phytoplankton species and obtained close values to the measured data, but the fitting curves exhibited some slight deviations under the low intensity of irradiance. Different phytoplankton species showed differences in photosynthetic productivity and characteristics. $P.\ subcordiformis$ showed larger intrinsic quantum yield ($\alpha$) and lower $I_{sat}$ and light compensation point ($I_c$) than $D.\ salina$ or $I.\ galbana$. $Microcystis$ sp., especially $M.\ aeruginosa$ with the largest $P_{nmax}$ and $\alpha$ among freshwater phytoplankton strains, exhibited more efficient light use efficiency than two species of green algae.

**Conclusions:** The present work will be useful both to describe the behavior of different phytoplankton in a quantitative way as well as to evaluate the flexibility and reusability of P-I models. Meanwhile we believe this research could provide important insight into the structure changes of phytoplankton communities in the aquatic ecosystems.

**Keywords:** Phytoplankton; Photosynthetic performance; irradiance; photosynthesis-irradiance response model
Background

Phytoplankton are a key functional component of aquatic ecosystems, play a pivotal role in biogeochemical cycles [1]. In particular, marine phytoplankton, as the principal driving force of ocean carbon cycles and energy flows, fix approximately 50 gigatons of inorganic carbon annually, almost half of the total global primary production [2, 3]. They show higher CO$_2$ fixation rates and higher biomass productivity than any other photosynthetic organism [3]. With increasing concentration of CO$_2$ concentrations in the atmosphere and growing climate warming, an accurate estimate of photosynthetic productivity of phytoplankton becomes ever more important for modelling primary production and structure changes of phytoplankton communities in aquatic ecosystems, especially eutrophic lakes (e.g., Taihu, Erie, Winnipeg lake) and estuaries (e.g., Yangtze River).

Clarifying the relationship between photosynthesis and irradiance is a basis to evaluate the growth performance of phytoplankton. Irradiance acts as a driving force in photosynthesis. The level of irradiance affects the growth, CO$_2$ fixation efficiency, carbon metabolism, and cell composition of photosynthetic organisms [4-8]. While extensive studies have been carried out and many insights have enriched the basis of phytoplankton physiology in recent decades [9-11], the relationship remains poorly understood for phytoplankton. High irradiance causes photoinhibition by the production of reactive oxygen species (ROS) and damages the function of the most light-sensitive complex PSII [5]. Irradiance availability affects phytoplankton community composition and is one of the key factors causing cyanobacteria blooms [12]. Resource competition theory shows that species with lower “critical light intensity” are often superior, such as *Microcystis* [13].
On the other hand, phytoplankton cells are rich in proteins, polysaccharides, lipids, vitamins, and polyunsaturated fatty acids, which have stirred up great attention as a promising potential feedstock for biofuel, nutraceuticals, animal and aquaculture feed production [10, 14]. Many species have been used for commercial development, such as Dunaliella salina, Isochrysis galbana, Spirulina (or Arthrospira), Haematococcus pluvialis, and Scenedesmus obliquus [2, 6, 10]. Almost all fishes, bivalve molluscs, and crustaceans primarily graze on phytoplankton to build immunity against diseases during their early larval stages [12]. However, large-scale production of phytoplankton has rarely been successful, with no more than 1 g DW L$^{-1}$ biomass that is mainly limited by the inefficiency of photosynthesis in high-cell density cultivation [11, 14, 15]. The photosynthetic parameters can be seen as indicators to achieve sustainable carbon assimilation and TAG accumulation in Isochrysis zhangjiangensis [8]. Therefore, accurately quantizing photosynthetic performance is crucial for more economical integration of production management and operation of industrial-scale phytoplankton culture systems [16].

The response curve of photosynthesis to irradiance ($P$-$I$) is frequently used to characterize photosynthetic performance by fitting experimental data (measured as oxygen evolution or carbon uptake) with $P$-$I$ models [17]. Obtained photosynthetic parameters, including the maximum net photosynthetic rate ($P_{\text{max}}$), the optimal intensity of irradiance ($I_{\text{sat}}$), and the dark respiration rate ($R_d$) can be regarded as indicator to evaluate the response of phytoplankton to meet environmental changes. A variety of $P$-$I$ models for phytoplankton have been established in the last few decades [18-30]. Although many recent models are suggested based to “old models” established in the 70s and 80s and have some contributions to improve, the most extensive application are still found in
those “old models” [31-35]. For example, an examination of the literature overwhelmingly reveals in excess of 1950 papers on the model proposed by Platt et al.[20]. This is most probably because they are simpler than those new models with complex parameters and any new model must take many years to be fully adopted. Higher plant and phytoplankton possess similar photosynthetic systems. Ye et al. developed a model for higher plants that parameterizes the core characteristics of the irradiance response, including solar energy absorption of photosynthetic pigment molecules, energy transfer, and electron transport between photosynthetic apparatuses[36]. This has been widely applied in rice, wheat, soybean, sunflower and other plants [37, 38].

The objective of this study was to determine the various relationships between the photosynthetic productivity of phytoplankton and irradiance intensity and investigate the reliability of P-I models to estimate the photosynthetic performance for phytoplankton. We selected the rather extensive range of phytoplankton, including three isolated from the ocean and four from lakes, to measure their photosynthetic oxygen evolution under different irradiance intensity. Obtained P-I data were fitted by using P-I model for quantization the photosynthetic performances. One P-I model for higher plants developed by Ye et al.[36] (it was represented as model 4 in this study) was first used to compare against three most widely applied models for phytoplankton (them were represented as models 1, 2 and 3 in this study).

Materials and methods

Phytoplankton cultivation

The three strains of marine phytoplankton (Isochrysis galbana, Dunaliella salina and Platymonus subcordiformis) isolated from East China Seas were grown aseptically in f/2 medium. The four strains of freshwater phytoplankton (Microcystis aeruginosa FACHB-
905, *Microcystis wesenbergii* FACHB-1112, *Scenedesmus obliquus* FACHB-116 and *Chlorococcum* sp. FACHB-1556) were purchased from the Freshwater Algae Culture Collection (FACHB-collection) of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China) and cultivated in BG11 medium. The cultures were illuminated by cool white fluorescent bulbs (60 µmol photons m\(^{-2}\) s\(^{-1}\)) with a photoperiod of 12 h per day at 26 ± 1 °C.

**Measurement of photosynthetic oxygen evolution**

After seven to ten days of incubation, the photosynthetic oxygen-evolving rate of microalgal cells reaching the exponential growth phase was determined using a bio-oxygen metre (Yaxin-1151, Beijing Yaxinliyi Science and Technology Co., Ltd., China). Eight-mL cell suspensions of each strain were exposed to increasing orders of irradiances (0, 25, 50, 100, 150, 200, 300, 400, 500, 600, 800, 1000, and 1200 µmol photons \(\text{m}^{-2}\) \(\text{s}^{-1}\)), given by a digital LED light source (YX-11LA, Beijing Yaxinliyi Science and Technology Co., Ltd., China), at 25 ± 1 °C. The metre took reads once every three seconds for 5 min in each irradiance measurement point, during which a linear relationship varying with time in oxygen concentration was obtained. Triplicate samples were prepared and measured for each test. The response of the photosynthetic oxygen-evolving rate to irradiance was fitted with four \(P-I\) models [18-20, 22, 36].

**Determination of chlorophyll a concentration and cell counts**

The cells for photosynthetic oxygen-evolving measurement were collected by centrifugation (5600 × g) for 10 min at 4 °C. Chlorophyll \(a\) (Chl \(a\)) was extracted from microalgal cells in 90% (v/v) acetone and left overnight at 4 °C in darkness. The extracts were then centrifuged at 3600 × g for 10 min. The Chl \(a\) concentration was determined spectrophotometrically in the supernatant with a SP752 UV-vis spectrophotometer.
(Spectrum Instruments, Shanghai, China) according to the method of Jeffrey & Humphrey [39]. One-mL cultures of each strain were taken and preserved in Lugol's iodine solution for counting algal cells by a haemocytometer. Each test was conducted in triplicate.

**Model description**

**Model 1**

The light dependence of the net photosynthetic rate ($P_n$) is expressed as [22]:

$$P_n = P_{n_{\text{max}}} \tanh \left( \frac{\alpha I}{P_{n_{\text{max}}}} \right) - R_d$$

where $P_n$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$) is the chlorophyll $a$-normalised net photosynthetic rate at irradiance $I$, $P_{n_{\text{max}}}$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$) is the light-saturated maximum rate of photosynthesis, $\alpha$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$/μmol photons m$^{-2}$ s$^{-1}$) is the light-limited initial slope, and $R_d$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$) is the dark respiration rate.

The saturation irradiance ($I_{\text{sat}}$, μmol photons m$^{-2}$ s$^{-1}$) corresponding to the light-saturated maximum rate ($P_{n_{\text{max}}}$) of photosynthesis is calculated as [1]:

$$I_{\text{sat}} = \frac{P_{n_{\text{max}}} - R_d}{\alpha}$$

But the analytic solution of the light compensation point ($I_c$, μmol photons m$^{-2}$ s$^{-1}$) can not be obtained by equation (1). In order to obtain $I_c$, Kok effect [40] must be ignored here, and $I_c$ can be calculated as [19]:

$$I_c = \frac{R_d}{\alpha}$$

The photosynthetic quantum efficiency ($P_n'$, μmol O$_2$ μmol photons$^{-1}$) is calculated as:

$$P_n' = \frac{\alpha}{\cosh^2 \frac{\alpha I}{P_{n_{\text{max}}}}}$$

**Model 2**

The light dependence of $P_n$ is expressed as [19, 20]:

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where \( P_n \) is the chlorophyll \( a \)-normalised net photosynthetic rate at irradiance \( I \); \( P_s \) is the parameter reflecting the maximum, potential, light-saturated, rate of photosynthesis; \( \alpha \) is the light-limited initial slope; \( \beta \) is the dimensionless parameter reflecting the photoinhibition process; and \( R_d \) is the dark respiration rate.

The \( I_{\text{sat}} \) is calculated as:

\[
I_{\text{sat}} = \frac{P_s}{\alpha} \ln \frac{\alpha + \beta}{\beta} \tag{6}
\]

The \( P_{\text{max}} \) can be calculated as:

\[
P_{\text{max}} = P_s \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{\beta}{\alpha + \beta} \right)^\frac{\beta}{\alpha} - R_d \tag{7}
\]

However, the analytic solution of \( I_c \) can not be obtained by equation (5). To obtain \( I_c \), the Kok effect must be ignored here, and \( I_c \) can be calculated as:

\[
I_c = \frac{R_d}{\alpha} \tag{8}
\]

The photosynthetic quantum efficiency is calculated as:

\[
P'_n = \exp \left( -\frac{\beta I}{P_s} \right) \left\{ \alpha \exp \left( -\frac{\alpha I}{P_s} \right) - \beta \left[ 1 - \exp \left( -\frac{\alpha I}{P_s} \right) \right] \right\} \tag{9}
\]

**Model 3**

The light dependence of \( P_n \) is expressed as [18]:

\[
P_n = \frac{I}{\alpha I^2 + \beta I + \gamma} - R_d \tag{10}
\]

Here \( P_n \) is the chlorophyll \( a \)-normalised net photosynthetic rate at irradiance \( I \); \( \alpha \) and \( \beta \) are the fundamental parameters, nondimensional; and \( R_d \) is the dark respiration rate. The reciprocal of \( \gamma \) is the light-limited initial slope.
\[ I_{\text{sat}} = \sqrt{\frac{\gamma}{\alpha}} \]  

(11)

\[ P_{\text{max}} \text{ is given by:} \]

\[ P_{\text{max}} = \frac{1}{\beta + 2\sqrt{\gamma \alpha}} - R_d \]  

(12)

When \( P_n = 0 \), \( I_c \) is given as follows,

\[ I_c = \frac{1 - \beta R_d + \sqrt{(1 - \beta R_d)^2 - 4 \gamma R_d}}{2 \alpha R_d} \]  

(13)

The photosynthetic quantum efficiency is calculated as:

\[ P_n' = \frac{\gamma - \alpha I^2}{(\gamma + \beta I + \alpha I^2)^2} \]  

(14)

Model 4

The light dependence of \( P_n \) is expressed as [36]:

\[ P_n = \alpha \frac{1 - \beta I}{1 + \gamma I} I - R_d \]  

(15)

Here \( P_n \) is the chlorophyll \( a \)-normalised net photosynthetic rate at irradiance \( I \), \( \alpha \) is the initial slope of the \( P_n-I \) response curve, \( \beta \) and \( \gamma \) are the nondimensional parameters reflecting photoinhibition and light saturation, respectively, and \( R_d \) is the dark respiration rate.

\( I_{\text{sat}} \) is calculated as:

\[ I_{\text{sat}} = \frac{\sqrt{(\beta + \gamma)}}{\gamma} - 1 \]  

(16)

\( P_{\text{max}} \) is obtained by:

\[ P_{\text{max}} = \alpha \left( \sqrt{\frac{\beta + \gamma}{\gamma}} - \frac{\beta}{\gamma} \right)^2 - R_d \]  

(17)

When \( P_n = 0 \), \( I_c \) is given as follows,

\[ I_c = \frac{\alpha - \gamma R_d + \sqrt{(\alpha - \gamma R_d)^2 - 4 \alpha \beta R_d}}{2 \alpha \beta} \]  

(18)
The photosynthetic quantum efficiency is calculated as:

\[ P'_n = \alpha \frac{1-2\beta I - \beta \gamma I^2}{(1+\gamma I)^2} \]  

(19)

**Statistical analysis**

\( P\)-\( I \) data were fitted using SPSS version 24.0 using nonlinear, least-squares fitting based on the Levenberg–Marquardt algorithm. Duncan’s post hoc tests (\( p < 0.05 \)) were performed to establish differences among fitted results from model 1, model 2, model 3 and model 4. Data were reported as the means and standard errors in the calculations. Goodness of fit of the mathematical models to experimental data was assessed using the adjusted coefficient of determination (\( R^2 \)).

**Results**

**Comparison of different P-I models of production curves**

Applying different values of the fundamental parameters to the model, the differences in the characteristics of production curves among model 2, model 3 and model 4 were compared, save for model 1, without consideration of light-inhibition at high irradiant intensity. Assuming that the initial slope \( \alpha \) was 0.5 (the initial slope of the curve equals the reciprocal of \( \gamma \) in model 3), increasing values of the light-saturated or photoinhibition parameters decreased \( P_{n\text{max}} \) of the curve and increased the magnitude of inhibition in three types (Fig. 1b-f), which indicated that they could closely reproduce the trend of the \( P_n\)-\( I \) curve. However, although \( P_s \) is defined as being associated with \( P_{n\text{max}} \) in model 2, the given value of \( P_s \) was over 30 ~ 125% of \( P_{n\text{max}} \), for which the biological implication is difficult to understand (Fig. 1a). However, in Fig. 1c, \( I_{\text{sat}} \) was kept constant value versus the change of \( \beta \) because \( I_{\text{sat}} \) was barely related to \( \alpha \) or \( \gamma \), according to Eqn. 16. In fact, greater \( \beta \) values were associated with greater bends of the curve, indicating saturation occurred more easily. Thus, Fig. 1c is clearly contradictory to the basis of photosynthetic
physiology.

The morphological and growth characteristics of phytoplankter

The morphology of the cultured cells was observed under a 600x optical microscope. Cells were mostly spherical, at 4.3 ~ 10 μm in diameter, and grew singly, except for S. obliquus. The Chl a contents were 1.647 ± 0.015, 2.778 ± 0.077, 2.297 ± 0.027, 1.320 ± 0.005, 1.739 ± 0.012, 1.318 ± 0.027 and 4.158 ± 0.077 mg L⁻¹ for cultures of I. galbana, D. salina, P. subcordiformis, M. aeruginosa, M. wesenbergii, S. obliquus and Chlorococcum sp., respectively (Table 1), which was used to normalize the photosynthetic oxygen-producing rate of phytoplankton. This normalization will reduce the variability of photosynthetic oxygen-producing rates as a result of differences in biomass, facilitating the comparison of photosynthetic performance. The Chl a content per cell of I. galbana, D. salina, P. subcordiformis, M. aeruginosa, M. wesenbergii, S. obliquus, and Chlorococcum sp. was 2.570 ± 0.042, 27.118 ± 1.151, 22.931 ± 0.563, 1.972 ± 0.044, 2.404 ± 0.031, 9.126 ± 0.600, and 4.578 ± 0.106 ng 10⁴ cells⁻¹, respectively.

P-I curve and P’-I curve of freshwater phytoplankton

The P-I curves for M. aeruginosa, M. wesenbergii, S. obliquus and Chlorococcum sp. are given in Fig. 3A. For almost all strains, $P_n$ increased rapidly with $I$ under low irradiance intensity, and reached saturation at 400 μmol photons m⁻² s⁻¹. $P_n$ exhibited a sharp decline for M. aeruginosa, M. wesenbergii, and S. obliquus yet only a slow decline for Chlorococcum sp. with the increasing $I$. As was observed for marine phytoplankton, all curves exhibited photoinhibition above the $I_{sat}$, but in addition to those estimated by model 1.

M. aeruginosa and M. wesenbergii are two different species of Microcystis sp., and
despite having nearly identical $P$-$I$ curves, there were some differences in the photosynthetic parameters obtained by the different models (Table 3). The values of $P_{\text{max}}$ obtained by models 2, 3 and 4 were close to their measured values (approximately 290.83 μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$ for $M. \text{aeruginosa}$ and 201.29 μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$ for $M. \text{wesenbergii}$), with < 5% of errors. Nevertheless, the values of $I_{\text{sat}}$ calculated by models 2 and 3 for $M. \text{aeruginosa}$ and $M. \text{wesenbergii}$ were far below their measured values, with significant differences ($p < 0.05$). For $S. \text{obliquus}$, the values of $P_{\text{max}}$ obtained by models 2, 3 and 4 were just under 1% of the measured value, yet all the corresponding $I_{\text{sat}}$ were over the measured value. The $P_{\text{max}}$ calculated by models 2, 3 and 4 for $\text{Chlorococcum sp.}$ were 75.25 ± 3.79, 76.15 ± 3.89 and 74.59 ± 4.23 μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$, respectively, while the $I_{\text{sat}}$ were 311.04 ± 17.27, 339.85 ± 15.19 and 396.06 ± 15.9 μmol photons m$^{-2}$ s$^{-1}$, respectively. No significant differences were found between the $I_{\text{sat}}$ calculated by model 4 and the measured data ($p < 0.05$). The photosynthetic parameters obtained by model 1 were still far from the measured data for these freshwater phytoplankton; above all, the $I_{\text{sat}}$ were seriously underestimated. For $\alpha$, the estimated by model 4 was the highest for all strains among the other three models. There were no significant differences in the estimation of $I_c$ or $R_d$ among each model. Fig. 3B indicates that the nonlinear change of $P'$ as $I$ in four species of freshwater phytoplankton was similar to that in marine phytoplankton.

**P-I curve and $P'$-I curve of marine phytoplankton**

The $P$-$I$ curves of $I. \text{galbana}$, $D. \text{salina}$ and $P. \text{subcordiformis}$ are shown in Fig. 2A, and obvious differences were observed among strains. $P_n$ increased gradually with $I$ towards saturation, which was at 800 μmol photons m$^{-2}$ s$^{-1}$ for $I. \text{galbana}$. However, for $D. \text{salina}$ and $P. \text{subcordiformis}$, $P_n$ increased steeply, almost linearly, within low
irradiance intensity (below 200 μmol photons m\(^{-2}\) s\(^{-1}\)), and it decreased rapidly when it reached the maximum value. All curves stopped above the \(I_{\text{sat}}\), excluding those produced by model 1, which indicates the presence of photoinhibition.

Differences were also observed in photosynthetic characteristic parameters calculated by the four types of models (Table 2). Model 1 either overestimated \(P_{\text{max}}\) or underestimated \(I_{\text{sat}}\), and these values showed significant differences with their measured values \((p < 0.05)\) for three strains of marine phytoplankton. The \(P_{\text{max}}\) obtained by models 2, 3 and 4 for \textit{I. galbana} were 97.45 ± 3.02, 97.55 ± 3.37 and 98.33 ± 3.20 μmol O\(_2\) mg\(^{-1}\) Chl \(a\) h\(^{-1}\), respectively. The \(I_{\text{sat}}\) corresponding to \(P_{\text{max}}\) were 709.60 ± 26.89, 699.26 ± 32.19, 766.17 ± 24.38 μmol photons m\(^{-2}\) s\(^{-1}\), respectively. Despite no significant differences in either estimated \(P_{\text{max}}\) or \(I_{\text{sat}}\) by the three models \((p > 0.05)\), model 4 fitted the values to the measured values with < 5% of errors. For \textit{D. salina}, the \(P_{\text{max}}\) estimated by models 2, 3 and 4 were 113.73 ± 6.24, 114.45 ± 6.24 and 113.31 ± 5.87 μmol O\(_2\) mg\(^{-1}\) Chl \(a\) h\(^{-1}\), respectively, while the \(I_{\text{sat}}\) obtained by model 2 and model 3 were notably lower than the measured value, with significant differences \((p < 0.05)\). The \(I_{\text{sat}}\) obtained by model 4 was 510.24 ± 2.92 μmol photons m\(^{-2}\) s\(^{-1}\), which was quite similar to the measured value (approximately 500 μmol photons m\(^{-2}\) s\(^{-1}\)). The values of \(P_{\text{max}}\) estimated by models 2, 3 and 4 for \textit{P. subcordiformis} were 94.64 ± 6.65, 95.59 ± 6.63, and 92.20 ± 6.56 μmol O\(_2\) mg\(^{-1}\) Chl \(a\) h\(^{-1}\), respectively; however, the calculated \(I_{\text{sat}}\) were significantly higher than the measured values \((p < 0.05)\), likely because of the rapid increase of \(P_n\) during low-intensity irradiance. The initial slope of the \(P-I\) curve \(a\), namely, the intrinsic quantum yield, estimated by model 4 was higher for all strains than those estimated by other models, with significant differences \((p < 0.05)\) for \textit{D. salina} and \textit{P. subcordiformis}.

The photosynthetic quantum yield represents the efficiency of carbon dioxide fixation
or oxygen evolution by a photosynthetic apparatus driven by absorbed photon energy, that is, the conversion efficiency of absorbed solar energy into chemical energy. Fig. 2B shows that the quantum yield calculated by models 2, 3 and 4 for *I. galbana*, *D. salina* and *P. subcordiformis* decreased as *I* increased, until it was equal to zero at the *I*\textsubscript{sat} point. Subsequently, it became negative as *I* increased, which also reveals why *P*\textsubscript{n} decreased as *I* increased above *I*\textsubscript{sat}. However, the values of *P*’\textsubscript{n} obtained by model 1 were always greater than zero with increasing *I* due to the asymptotic function in this model.

**Discussion**

Photosynthesis is not only a biochemical process achieved by photosynthetic apparatuses, it also contains a biophysical process [5, 9, 41]. As shown in Fig. 4, photosynthetic pigment molecules (*Chl*), such as Chlorophyll *a* and *b* and carotenoids, absorb solar energy, which induces them into an excited state (*Chl*\*). The largest amount of exciton binding energy is transferred to the photochemical reaction centres (*P*\textsubscript{680} and *P*\textsubscript{700}), where charge separation occurs and produces electrons (*e*\textsuperscript{−}) and accompanied by the splitting of water into *P*\textsubscript{680*}. Other energy is transformed into fluorescence and heat [5, 17, 25, 28, 29]. *Chl*\* conducts de-excitation by photochemistry, non-radiation heat dissipation, and chlorophyll fluorescence then able to accept new photons, yet the process depend on the lifetime of *Chl* in the excited state [41, 42]. The released electrons pass through pheophytin to the first electron acceptor *Q*\textsubscript{A} and are ultimately transferred via a series of electron carriers to photosystem I, thereby producing ATP and reducing NADPH to driving photosynthetic carbon fixation and respiratory carbon oxidation [5, 26]. Although a variety of *P*-*I* models have been established and used to fit the *P*-*I* curve for estimating photosynthetic performance and responses to environment changes for phytoplankton [18-20, 22-30, 43], many of them were not built based on the
photosynthetic mechanism.

The exponential model established by Webb et al. [23] and model 1 are still applied extensively for phytoplankton [31-35] even though they lack photoinhibition function. For example, Ma et al. [34] indicated that the $P_{\text{max}}$ calculated by model 1 for $M. \text{aeruginosa}$ FACHB-905 and $M. \text{aeruginosa}$ FACHB-469 were $253.92 \pm 6.79$ and $231.32 \pm 6.40$ μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$, respectively, at 25 °C, yet the corresponding $I_{\text{sat}}$ were only $92.71 \pm 7.86$ and $88.61 \pm 3.22$ μmol photons m$^{-2}$ s$^{-1}$, respectively. Furthermore, the shape of their $P$-$I$ curves did not appear to decline above $I_{\text{sat}}$. In our study, the values of $a$, $P_{\text{max}}$ and $I_{\text{sat}}$ fitted by model 1 showed significant differences with those obtained by other models ($p < 0.05$); either $P_{\text{max}}$ or $I_{\text{sat}}$ were distinct from their measured data for seven strains of phytoplankton (including $M. \text{aeruginosa}$ FACHB-905), which suggests that an insufficient irradiance would be supplied to the cultivation if the $I_{\text{sat}}$ was used as the optimal intensity of irradiance.

To describe the entire range of light levels of phytoplankton, Platt et al. [19, 20] proposed another empirical model with a photoinhibition function (model 2 in this study). Superficially, the $P$-$I$ curves fitted by model 2 seem to be perfect as other studies [44, 45], but the value of $P_s$ among the fitted results was notably higher than the value of $P_{\text{max}}$ in seven phytoplankton strains, whether $\beta > 0$ or $\beta = 0$ (Table 4). However, $P_{\text{max}} = P_s$ by Eqn. 7, where there was no inhibition at $\beta = 0$, and the fitting curves were similar to model 1 (Fig. 6). Additionally, $P_s$ appeared to fluctuate at $\beta > 0$, which indicates the presence of inhibition among $I. \text{galbana}$, $M. \text{aeruginosa}$, $M. \text{wesenbergii}$ and $S. \text{obliquus}$. This reveals a clear disagreement with the definition of $P_s$ that characterizes the output of dark reactions of photosynthesis in model 2. Therefore, improvement of model 2 is needed to redefine the biological implication of some fundamental parameters according to the
Compared with previous models, model 3 is no longer just a mathematical equation describing the dependence of the photosynthetic rate on irradiance intensity. Its foundation is an assumption of “photosynthetic factories” (PSF) on physiological mechanisms proposed by Crill [21]. A PSF that is regarded as a combination of photosystem I (PSI) and PSII conducts one unit of light to generate one unit of photosynthetic product. And Eilers and Peeters assumed that the process of photosynthesis is modeled by changes of the states of PSF from the resting state to the activated and inhibited state [17, 18]. Model 3 yielded a good-fitting curve for the $P$-$I$ data of all strains of phytoplankton in this study, and the returned values for $P_{\text{max}}$, $I_c$, and $R_d$ were close to their measured values, except for $I_{\text{sat}}$, which showed a large deviation ($p < 0.05$). Meanwhile, Fig. 1c shows that the $I_{\text{sat}}$ of curve did not change with the value of $\beta$. This may be because there is no assumption of the capture of solar energy, energy transfer process, or electron transport process from PSII to Cytb6f and then to PSI.

Although differences between higher plants and phytoplankton are observed in photosynthetic antenna system and photosynthetic components [10, 16], in present study the $P$-$I$ curves of all phytoplankton species fitted by model 4, which be developed for higher plants, were good and the returned values were also close to the measured data. This reveals that $P$-$I$ models for higher plants are applicable for phytoplankton. Acquiring an accurate and optimal parameter for irradiance intensity is essential to achieve high biomass of phytoplankton in production. Irradiance is rapidly attenuated during high-cell density cultivation of phytoplankton [14, 25]. Variation in the pigment composition of light harvesting complexes with irradiance intensity has been observed in most species of phytoplankton [4, 5]. Irradiance intensity also regulates the accumulation of photosynthesis mechanism.
Note that obtained $I_{sat}$ by model 4 was closer to the measured value than other three models. The differences between the returned values for $P_{nmax}$, $I_c$, and $R_d$ by model 4 and their measured values were slightly larger than those by model 3, without significant differences ($p > 0.05$). The fitting curves by model 4 for *P. subcordiformis*, *M. aeruginosa*, *M. wesenbergii*, and *Chlorococcum* sp. exhibited some deviations under low intensity of irradiance, likely because the model targeted higher plants, which showed higher light dependence than phytoplankton.

In meso- and eutrophic water bodies, irradiance or temperature is a key factor affecting changes of phytoplankton community composition, especially for those that become the dominant population between cyanobacteria and green algae [46]. The results of this study explicitly demonstrate that *M. aeruginosa* and *M. wesenbergii* had high intrinsic quantum efficiency ($\alpha$), while their Chl $a$ content per cell was lower than that of both *S. obliquus* and *Chlorococcum* sp., indicating the efficient light harvesting and use for *M. aeruginosa* and *M. wesenbergii*. In addition, almost two times less $\alpha$ than both *S. obliquus* and *Chlorococcum* sp., and the largest $P_{nmax}$ were found in *M. aeruginosa*. However, *M. aeruginosa* is the main contributor of notorious bloom-forming cyanobacteria in global freshwater bodies, such as Dianchi Lake in China [47]. These results reveal the underlying physiological basis of photosynthesis of *Microcystis* with lower “critical light intensity”, and provide important insights into the management and control of cyanobacteria in changing lakes and estuarine waters.

*I. galbana* and *D. salina* are applied world-wide to generate biofuels due to their rich lipids (lipid levels between 23 and 55% by weight of dry biomass), and they are also commonly cultivated with *P. subcordiformis* (lipid levels between 20 and 30% by weight of dry biomass) for aquaculture in China, Japan, Australia, and southeast Asia [14, 48].
To meet nutritional requirements, mixed cultures of two or more species of phytoplankton are often fed to larvae in seed fanning of aquatic products [49]. It is critical that the photosynthetic productivity of each strain reach as high as possible during production. The comparison revealed that, although the $P_{\text{max}}$ lay between $I$. galbana and $P. subcordiformis$, other photosynthetic characteristic parameters showed great differences. The smallest $\alpha$ and highest $I_c$ were found in $I$. galbana, which meant a low efficiency of light capture and use for $I$. galbana because the intrinsic quantum yield represents the numbers of photosynthetic electrons required to assimilate one CO$_2$ molecule [8]. In contrast, the largest $\alpha$ and lowest $I_{\text{sat}}$ and $I_c$ were in $P. subcordiformis$, although it possesses lower Chl $a$ content per cell than that of $D. salina$. Consequently, the ranking of light-dependence in descending order was $P. subcordiformis, D. salina, and I. galbana. Under co-culture conditions, a gradient of irradiance from low to mid to high can be supplied in one photoperiod.

**Conclusions**

Our study showed that significant differences were found between the returned values to photosynthetic characteristics by models 1, 2 and 3, some parameters (e.g., $I_{\text{sat}}$) were distinctly different to the measured data. Model 4 for higher plants reproduced the irradiance response trends of photosynthesis well, was applicable for phytoplankton, but more studies are required to investigate its flexibility and reusability. Differences in photosynthetic performance were observed among phytoplankton species. $P. subcordiformis$ showed higher light-dependence than $D. salina$ and $I. galbana$, while $M. aeruginosa$ and $M. wessenbergii$ exhibited more efficient light use than $S. obliquus$ and *Chlorococcum* sp.. These findings could contribute to a better understanding of structure changes of phytoplankton communities in the aquatic ecosystem, especially in those
eutrophic lakes and estuaries.

List of abbreviations
Photosystem I (PSI);
Photosystem II (PSII);
Reactive oxygen species (ROS);
Response of photosynthesis to irradiance (P-I);
Photosynthetic factories (PSF);
Chlorophyll a (Chl a);
Irradiance intensity ($I$, $\mu$mol photons m$^{-2}$ s$^{-1}$);
Net photosynthetic rate at irradiance $I$ ($P_n$, $\mu$mol O$_2$ mg$^{-1}$ Cha h$^{-1}$);
Maximum net photosynthetic rate ($P_{n\text{max}}$, $\mu$mol O$_2$ mg$^{-1}$ Cha h$^{-1}$);
Saturation irradiance ($I_{\text{sat}}$, $\mu$mol photons m$^{-2}$ s$^{-1}$);
Light-limited initial slope ($\alpha$, $\mu$mol O$_2$ mg$^{-1}$ Cha h$^{-1}$/$\mu$mol photons m$^{-2}$ s$^{-1}$);
Light compensation point ($I_c$, $\mu$mol photons m$^{-2}$ s$^{-1}$);
Dark respiration rate ($R_d$, $\mu$mol O$_2$ mg$^{-1}$ Cha h$^{-1}$);
Adjusted coefficient of determination ($R^2$);
Photosynthetic quantum efficiency ($P_n'$, $\mu$mol O$_2$ $\mu$mol photons$^{-1}$);
Response of photosynthetic quantum efficiency to irradiance ($P_n'$-$I$);
Parameter reflecting the maximum, potential, light-saturated, rate of photosynthesis in model 2 ($P_s$);
Photosynthetic pigment molecules (Chl);
Excited state of photosynthetic pigment molecules (Chl*).
Ethics approval and consent to participate
Not applicable

Consent for publication
All authors consented to the publication of this work.

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article.

Competing interests
The authors declare no competing interests.

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Authors’ contributions
XLY conceived the original study, wrote the paper. XLY and LHL performed the experiment and data analysis. XYW and ZKY conducted the isolation and identification of marine phytoplankton. SBW and ZPY supervised the experiment and editing of paper. All authors read and approved the manuscript.

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Table and Figure captions

**Table 1** The Chlorophyll *a* content and cell number profiles of seven phytoplankton cultures.

**Table 2** Comparison of the results fitted by Models 1, 2, 3 and 4 with measured data in marine phytoplankton.

**Table 3** Comparison of the results fitted by Models 1, 2, 3 and 4 with measured data in freshwater phytoplankton.

**Table 4** Comparison of *P*ₚ and *P*ₙₘₐₓ (μmol O₂·mg⁻¹ Cha·h⁻¹) calculated by model 2 with measured values.

**Fig. 1** Model 2, Model 3 and Model 4 responses of the net photosynthetic rate (*P*ₚ) versus irradiance intensity (*I*) determined for the different values of the fundamental parameters, respectively. (a) and (b) were obtained by Model 2, (c) and (d) were obtained by Model 3, and (e) and (f) were obtained by Model 4.

**Fig. 2** The *P*-*I* curves (A) and *P*’*-I* curves (B) of *Isochrysis galbana*, *Dunaliella salina* and *Platymonas subcordiformis*.

**Fig. 3** The *P*-*I* curves (A) and *P*’*-I* curves (B) of *Microcystis aeruginosa*, *Microcystis wesenbergii*, *Scenedesmus obliquus* and *Chlorococcum* sp..

**Fig. 4** Schematic representation of the mechanism of photosynthesis consisting of biophysical and biochemical processes.

**Fig. 5** The *P*-*I* curves produced by the model 2 at β = 0 and β > 0.
Table 1 The Chlorophyll $a$ content and cell number profile of seven phytoplankton cultures.

| Strains                        | Chl $a$ (mg L$^{-1}$) | Cell density ($10^5$ cells mL$^{-1}$) | Cell size (μm) |
|--------------------------------|-----------------------|----------------------------------------|----------------|
| Isochrysis galbana             | 1.647±0.015           | 641.00±5.95                            | 5.8±0.4        |
| Dunaliella salina              | 2.778±0.077           | 103.00±7.00                            | 9.8±0.2        |
| Platymonas subcordiformis      | 2.297±0.027           | 100.33±3.38                            | 10.0±0.1       |
| Microcystis aeruginosa FACHB-905 | 1.320±0.005          | 669.67±12.35                           | 4.3±0.4        |
| Microcystis wesenbergii FACHB-1112 | 1.739±0.012      | 723.33±5.18                            | 5.1±0.2        |
| Scenedesmus obliquus FACHB-116  | 1.318±0.027           | 145.33±6.64                            | 8.1±0.5        |
| Chlorococcum sp. FACHB-1556    | 4.158±0.077           | 908.67±14.08                           | 6.0±1.2        |

Table 2 Comparison of results fitted by Model 1, 2, 3 and 4 with measured data in marine phytoplankton.

| Models                        | Photosynthetic parameters |  |  |  |  |  |
|-------------------------------|----------------------------|---|---|---|---|---|
|                              | $a$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$) & $P_{max}$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$) & $I_{act}$ (μmol photons m$^{-2}$ s$^{-1}$) & $I_{c}$ (μmol photons m$^{-2}$ s$^{-1}$) & $R_{s}$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$) | $R^2$ |
| Isochrysis galbana            |                            |   |   |   |   |   |
| Model 1                       | 0.411±0.032$^a$            | 119.51±4.72$^a$                     | 229.90±13.60$^a$                     | 63.12±2.33$^a$                      | 25.97±2.54$^a$                     | 0.981±0.007$^a$                     |
| Model 2                       | 0.468±0.037$^a$            | 97.45±3.02$^b$                      | 709.60±26.89$^a$                     | 56.71±2.41$^a$                      | 25.97±2.60$^a$                     | 0.986±0.007$^a$                     |
| Model 3                       | 0.373±0.020$^b$            | 97.55±3.37$^b$                      | 699.26±32.19$^b$                     | 65.51±3.48$^b$                      | 23.98±1.95$^b$                     | 0.989±0.007$^b$                     |
| Model 4                       | 0.482±0.037$^a$            | 98.33±3.20$^b$                      | 766.17±24.38$^b$                     | 61.06±2.82$^b$                      | 26.63±2.44$^a$                     | 0.986±0.008$^a$                     |
| Measured                      | ≈ 94.14                    | ≈ 800                                | ≈ 62                                | ≈ 24.29                            |                                             |                                             |
| Dunaliella salina             |                            |   |   |   |   |   |
| Model 1                       | 0.874±0.023$^c$            | 123.09±5.88$^a$                     | 116.90±23.87$^c$                     | 23.87±1.66$^c$                      | 20.92±1.97$^c$                     | 0.944±0.016$^c$                     |
| Model 2                       | 1.006±0.033$^b$            | 113.73±6.24$^b$                     | 453.39±6.87$^b$                      | 21.27±1.75$^a$                      | 21.39±1.81$^a$                     | 0.990±0.001$^a$                     |
| Model 3                       | 0.918±0.058$^{bc}$         | 114.45±6.24$^a$                     | 444.33±6.04$^a$                      | 23.09±2.29$^a$                      | 19.89±1.14$^a$                     | 0.989±0.007$^a$                     |
| Model 4                       | 1.202±0.037$^a$            | 113.31±5.87$^a$                     | 510.24±2.92$^a$                      | 21.67±1.93$^a$                      | 23.30±1.93$^a$                     | 0.983±0.002$^a$                     |
| Measured                      | ≈ 119.24                   | ≈ 500                                | ≈ 23                                | ≈ 20.25                            |                                             |                                             |
| Platymonas subcordiformis     |                            |   |   |   |   |   |
| Model 1                       | 1.975±0.055$^d$            | 107.96±5.58$^a$                     | 41.25±1.62$^d$                      | 13.40±2.21$^a$                      | 26.32±3.93$^a$                     | 0.883±0.010$^c$                     |
| Model 2                       | 2.479±0.023$^c$            | 94.64±6.65$^a$                      | 212.36±7.80$^c$                      | 11.05±1.65$^a$                      | 27.45±4.30$^a$                     | 0.975±0.009$^a$                     |
| Model 3                       | 2.834±0.056$^b$            | 95.59±6.63$^d$                      | 251.97±9.73$^c$                      | 10.82±1.87$^b$                      | 26.13±4.26$^b$                     | 0.958±0.013$^b$                     |
| Model 4                       | 3.640±0.031$^a$            | 92.20±6.56$^b$                      | 299.55±10.72$^a$                     | 9.68±1.71$^a$                       | 28.36±4.21$^a$                     | 0.934±0.013$^b$                     |
| Measured                      | ≈ 100.13                   | ≈ 150                                | ≈ 14                                | ≈ 24.49                            |                                             |                                             |
Table 3 Comparison of results fitted by Model 1, 2, 3 and 4 with measured data in freshwater phytoplankton.

| Models          | Photosynthetic parameters |
|-----------------|---------------------------|
|                 | $a$ ($\mu$mol O$_2$ mg$^{-1}$ Chl a h$^{-1}$/µmol photons m$^{-2}$ s$^{-1}$) | $P_{max}$ ($\mu$mol O$_2$ mg$^{-1}$ Chl a h$^{-1}$) | $I_{sat}$ ($\mu$mol photons m$^{-2}$ s$^{-1}$) | $I_c$ ($\mu$mol photons m$^{-2}$ s$^{-1}$) | $R_d$ ($\mu$mol O$_2$ mg$^{-1}$ Chl a h$^{-1}$) | $R^2$ |
| Microcystis aeruginosa |                           |                                 |                                 |                                 |                                 |       |
| Model 1         | 2.404±0.103$^bc$          | 260.46±10.72$^a$                | 97.83±2.42$^a$                  | 10.57±1.12$^a$                  | 25.63±3.86$^a$                  | 0.721±0.047$^b$  |
| Model 2         | 2.416±0.074$^bc$          | 290.74±15.09$^a$                | 380.17±4.89$^b$                | 9.47±1.05$^a$                   | 22.92±2.75$^a$                  | 0.973±0.010$^a$  |
| Model 3         | 1.770±0.026$^bc$          | 296.37±14.89$^b$                | 340.82±4.49$^c$                | 10.26±2.54$^c$                  | 18.23±4.61$^c$                  | 0.969±0.015$^a$  |
| Model 4         | 2.967±0.067$^ac$          | 283.55±14.53$^c$                | 415.25±2.33$^a$                | 9.53±1.04$^a$                   | 26.86±2.75$^a$                  | 0.964±0.007$^a$  |
| Measured        | ≈ 290.83                  | ≈ 400                          | ≈ 10                           | ≈ 18.27                         |                               |       |
| Microcystis wesenbergii |                        |                                 |                                 |                                 |                                 |       |
| Model 1         | 1.879±0.039$^bc$          | 184.72±2.57$^c$                | 82.57±2.56$^c$                  | 15.85±1.68$^a$                  | 29.73±2.91$^bc$                 | 0.758±0.031$^b$  |
| Model 2         | 1.920±0.030$^bc$          | 195.32±1.50$^bc$                | 352.20±7.29$^b$                | 14.70±1.87$^a$                  | 28.15±2.44$^bc$                 | 0.974±0.006$^a$  |
| Model 3         | 1.309±0.074$^bc$          | 201.37±2.94$^c$                | 322.50±6.96$^c$                | 15.50±2.37$^c$                  | 20.46±3.90$^c$                  | 0.978±0.007$^a$  |
| Model 4         | 2.474±0.071$^a$           | 188.62±2.31$^bc$                | 389.62±9.62$^a$                | 14.81±1.47$^a$                  | 33.19±2.31$^a$                  | 0.954±0.011$^a$  |
| Measured        | ≈ 201.29                  | ≈ 400                          | ≈ 15                           | ≈ 23.73                         |                               |       |
| Scenedesmus obliquus |                      |                                 |                                 |                                 |                                 |       |
| Model 1         | 1.499±0.019$^bc$          | 265.88±5.70$^a$                | 159.64±1.84$^d$                | 17.82±3.32$^a$                  | 26.61±4.67$^a$                  | 0.912±0.006$^b$  |
| Model 2         | 1.581±0.010$^bc$          | 268.80±5.21$^a$                | 527.39±8.93$^b$                | 15.24±3.18$^a$                  | 24.05±4.91$^a$                  | 0.989±0.002$^a$  |
| Model 3         | 1.268±0.030$^bc$          | 268.70±5.25$^a$                | 481.60±8.51$^c$                | 15.34±3.74$^c$                  | 19.25±4.59$^c$                  | 0.989±0.001$^a$  |
| Model 4         | 1.751±0.021$^a$           | 268.11±4.98$^a$                | 561.94±8.40$^a$                | 15.82±3.15$^a$                  | 26.29±4.79$^a$                  | 0.987±0.002$^a$  |
| Measured        | ≈ 267.37                  | ≈ 400                          | ≈ 15                           | ≈ 19.91                         |                               |       |
| Chlorococcum sp. |                  |                                 |                                 |                                 |                                 |       |
| Model 1         | 0.979±0.007$^bc$          | 83.86±4.98$^a$                  | 68.66±4.47$^c$                 | 17.00±1.80$^a$                  | 16.66±1.82$^a$                  | 0.935±0.009$^c$  |
| Model 2         | 1.190±0.020$^bc$          | 75.25±3.79$^a$                  | 311.04±17.27$^b$               | 14.59±1.52$^a$                  | 17.43±2.09$^a$                  | 0.984±0.001$^b$  |
| Model 3         | 1.247±0.022$^bc$          | 76.15±3.89$^a$                  | 339.85±15.19$^b$               | 15.02±1.76$^b$                  | 16.82±2.09$^b$                  | 0.979±0.002$^b$  |
| Model 4         | 1.572±0.024$^a$           | 74.59±4.23$^a$                  | 396.06±15.93$^c$               | 13.87±1.59$^a$                  | 18.64±2.18$^a$                  | 0.964±0.001$^b$  |
| Measured        | ≈ 76.06                   | ≈ 400                          | ≈ 15                           | ≈ 15.03                         |                               |       |
Table 4 Comparison of $P_s$, $P_{\text{max}}$ (μmol O$_2$ mg$^{-1}$ Chl $a$ h$^{-1}$) calculated by model 2 with measured value

| Parameter | Isochrysis | Dunaliella | Platymonus | Microcystis aeruginosa | Microcystis wesenbergii | Scenedesmus obliquus | Chlorococcum sp. |
|-----------|------------|------------|------------|------------------------|------------------------|---------------------|-----------------|
| $P_s$ ($\beta = 0$) | 135.44±6.59 | 144.28±10.86 | 133.39±6.73 | 328.48±18.27 | 235.77±3.70 | 311.42±7.37 | 96.21±5.71 |
| $P_s$ ($\beta > 0$) | 14188.6±13735.8 | 196.08±31.06 | 135.23±7.45 | 1163.4±615.5 | 415.53±29.50 | 943.8±282.4 | 107.02±5.53 |
| $P_{\text{max}}$ | 97.45±3.02 | 113.73±6.24 | 94.64±6.65 | 290.74±15.09 | 195.32±1.50 | 268.80±5.21 | 75.25±3.79 |
| Observations | ≈ 94 | ≈ 119 | ≈ 100 | ≈ 290 | ≈ 200 | ≈ 267 | ≈ 75 |
Fig. 1 Model 2, Model 3 and Model 4 responses of the net photosynthetic rate ($P_n$) versus irradiance intensity ($I$) determined for different values of the fundamental parameters, respectively. (a) and (b) were obtained by Model 2, (c) and (d) were obtained by Model 3, (e) and (f) were obtained by Model 4.

Fig. 2 The $P-I$ curves (A) and $P'-I$ curves (B) in *Isochrysis galbana*, *Dunaliella salina* and *Platymonus*.
**Fig. 3** The $P-I$ curves (A) and $P'-I$ curves (B) in *Microcystis aeruginosa*, *Microcystis wesenbergii*, *Scenedesmus obliquus* and *Chlorococcum* sp.

**Fig. 4** Schematic representation of mechanism of photosynthesis consisted of biophysical and biochemical processes.
Fig. 5 The P-I curves produced by the model 2 at $\beta = 0$ and $\beta > 0$. 