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Multi-component kinetics for the growth of the cyanobacterium *Synechocystis* sp. PCC6803

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ABSTRACT

The growth kinetics of phototrophic microorganisms can be controlled by the light irradiance, the concentration of an inorganic nutrient, or both. A multi-component kinetic model is proposed and tested in novel batch experiments that allow the kinetic parameters for each factor to be estimated independently. For the cyanobacterium *Synechocystis* sp. PCC6803, the estimated parameters are maximum specific growth rate (μ_{max}) = 2.8/d, half-maximum-rate light irradiance (K_L) = 11 W/m^2, half-inhibition-rate light irradiance ($K_{L,I}$) = 39 W/m^2, and half-maximum-rate concentration for inorganic carbon ($K_{S,Ci}$) = 0.5 mgC/L, half-maximum-rate concentration for inorganic nitrogen ($K_{S,Ni}$) = 1.4 mgN/L, and half-maximum-rate concentration for inorganic phosphorus ($K_{S,Pi}$) = 0.06 mgP/L. Compared to other phototrophs having μmax estimates, PCC6803 is a fast-growing r-strategist relying on reaction rate. Its half-maximum-rate and half-inhibition rate values identify the ranges of light irradiance and nutrient concentrations that PCC6803 needs to achieve a high specific growth rate to be a sustainable bioenergy source. To gain the advantages of its high maximum specific growth rate, PCC6803 needs to have moderate light illumination (7–62 W/m^2 for $\mu_{syn} \geq$ 1/d) and relatively high nutrient concentrations: $N_i \geq$ 2.3 mgN/L, $P_i \geq$ 0.1 mgP/L, and $C_i \geq$ 1.0 mgC/L.

Keywords: Kinetics, Light irradiance, Nutrients, Photoautotrophy, *Synechocystis* sp. PCC6803

1. Introduction

Increasing restrictions on carbon dioxide (CO_2) discharges and depletion of petroleum reserves warrant attention to utilizing photoautotrophic microorganisms as a means to convert CO_2 into biofuels and biomaterials [1-3]. A practical objective is to maximize the biomass-production rate, since the biomass embodies the sunlight energy captured by photosynthesis. Although the photosynthetic pathways in photoautotrophs are complex [4], attempting to embody all of the metabolic details is intractable for practical application to PBR design and operation. Instead, a kinetic representation that captures the core mechanisms of photoautotrophic growth is essential and efficient for design and control of PBRs.

Traditional growth kinetics of photoautotrophs has focused mainly on limitation by light irradiance (LI) [5], but such modeling is not accurate when essential nutrients (i.e., inorganic carbon (C_i), nitrogen (N_i), and phosphorus (P_i)) limit the growth rate. Even though some previous work addressed the effects of LI, C_i, N_i and P_i [6], most previous work has not attempted to identify which component (or components) is (are) rate limiting and then to quantify the kinetic response to all major limiting components.

Another unique feature of photoautotrophs is that their overall rate of photosynthesis can be inhibited by excessive LI [7]. This situation is analogous to self-inhibition kinetics for certain organic and inorganic substrates [8]. In contrast, Monod kinetics can be used to represent photosynthetic growth kinetics for individual nutrients [9].

A multiplicative kinetic model appears frequently for dual limitation by the electron donor and electron acceptor for chemotrophic microorganisms [10]. However, this multiplicative model is not directly relevant to photosynthetic microorganisms, which produce energy and reducing power from photophosphorylation and use water as an electron donor; for example, water has a fixed and essentially unlimited concentration. In addition, inorganic nutrients are anabolic building blocks essential for synthesis processes. Even when they need to be reduced (e.g., for CO_2 and NO_3^-), they are reduced for anabolism, not respiration.

To capture these special features of phototrophic microorganisms, we propose a modified hybrid form of a multiplicative kinetic model for the specific growth rate in photosynthesis:

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$$\mu_{syn} = \mu_{\max} \cdot \frac{LI}{K_L + LI + LI^2/K_{LI}} \cdot \left[\frac{C_i}{K_{S,Ci} + C_i}, \frac{N_i}{K_{S,Ni} + N_i}, \frac{P_i}{K_{S,Pi} + P_i}, \cdots \right]_{\min} \quad (1)$$

where μ_{syn} is the specific growth rate, μ_{max} is the maximum specific growth rate, K_S is half-maximum-rate concentration for a given nutrient, K_L is the half-maximum-rate light irradiance, $K_{L,I}$ is the half-inhibition-rate light irradiance, LI is the light illumination to which the microorganisms are exposed, $K_{S,Ci,}$ is the half-maximum-rate concentrations for inorganic carbon, $K_{S,Ni}$ is the half-maximum-rate concentrations for inorganic nitrogen, $K_{S,Pi}$ is the half-maximum-rate concentrations for inorganic phosphorus, C_i is the total concentration of the carbon, N_i is the total concentration of the nitrogen, P_i is the total concentration of the phosphorus, and square bracket with subscript 'min' indicates the minimum value among the individual mathematical expressions separated by commas.

The approach of Eq. (1) expresses that LI drives the oxidation of H_2O to produce high-energy electrons in the form of nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine- triphosphate (ATP), while synthesis of new biomass is the major sink for the electrons and ATP. The specific growth rate for synthesis is controlled by LI according to self-inhibition kinetics, the first term in Eq. (1). As long as sufficient NAD(P)H and ATP are available from photosynthesis, the synthesis growth rate is controlled solely by the most-limiting inorganic nutrient, the second term in Eq. (1), which follows single-Monod kinetics, consistent with a literature [6]. If LI and a nutrient are concurrently rate limiting, the rate of production of phototrophic-biomass is governed by the multiplicative effect of light intensity (providing the electrons and ATP) and one most-limiting nutrient (controlling synthesis, the sink for the electrons and ATP), as shown by all of Eq. (1).

In determining the specific growth rate using Eq. (1), the locally available LI inside the PBR becomes essential, since LI varies with the length of the light path and biomass concentration according to the Beer-Lambert law [11]. It is convenient to compute an average LI integrated over the entire volume of the PBR [12], as this provides a single value for LI, which we call LI_{SA}, as it is the spatially averaged value. The contents of PBRs normally are well mixed so that the photosynthetic microorganisms are exposed to LI_{SA} on average. Thus, the multiplicative form captures that LI is readily attenuated in most experimental conditions by either the system's boundary or the biomass itself.

The primary objectives of this study are to evaluate the hybrid multiplicative model (Eq. (1) using LI_{SA}) for photoautotroph synthesis and to estimate the kinetic parameters for each of LI_{SA}, C_i, N_i, and P_i. We do so by using the cyanobacterium *Synechocystis* sp. PCC6803 and a specially designed series of batch experiments.

Synechocystis sp. PCC6803 is a good model photoautotroph, since it can achieve high biomass yield and is robust for wide ranges of temperature, salinity, and pH [13]. Moreover, the simple genetic structure of PCC6803 provides an opportunity to improve physiological characteristics through genetic manipulations [14]. Whether PCC6803 is wild type or genetically modified, enhancing its productivity will depend on identifying and quantifying the factors that limit its growth-rate in a photobioreactor (PBR) since ultimate goal is characterizing how to synthesize more biomass within shorter period of time.

We designed special batch experiments to allow the kinetic parameters for each factor to be estimated independently. We considered light attenuation to compute LI_{SA} inside the bioreactor and used LI_{SA} whenever LI was limiting. We evaluated the multiplicative connection between LI and the limiting nutrient, as well as self-inhibition by LI_{SA}. With estimates for all the kinetic parameters, we are able to assess the conditions that lead to a significantly high specific growth rate of PCC6803, $\geq$ 1/d.

2. Materials and Methods

2.1. Batch-Experiment Set Up

All batch experiments were conducted in 125-mL serum bottles (Wheaton Science Products, USA) made of clear borosilicate glass and with a liquid volume of 100 mL. The experimental set up is shown schematically in Fig. S1 of Supporting Information. The mouth was sealed with a snap-on rubber stopper and an aluminum cap after filling the bottle with medium. Gas-flow controllers, connected to an air pump (KNF Neuberger, USA) with an air-filter (0.2 mm PTFE; Whatmann, USA) inlet, regulated the supply rate of CO_2 by maintaining an aeration rate of 5 $L_{air}/L_{reactor}$/min through a tube inserted into the bottle through the rubber cap. The rapid gas bubbling make the liquid contents completely mixed, and off gas was vented out through a syringe needle while preventing mass transfer limitation of C_i.

The batch experiments were performed in a photo-incubation chamber (TC30; Conviron Inc., USA) maintained at 30°C. Light panels inside the chamber were equipped with white-fluorescent lamps and supplied photosynthetically active radiation (PAR)[15] with a controllable LI in the range of 3 - 20 W/m^2 (14 – 92 mmol photons/s/m^2) at the outside of the bottle. For attaining LI levels up to 48 W/m^2 (221 mmol photons/s/m^2), additional lamps were 92 installed. The PAR LI was measured with a PAR sensor (LI-190 Quantum sensor; LI-COR Biosciences, USA) connected to a digital multi-meter; thus, all the LI values reported in this study are on a PAR basis. The photon flux was converted to energy flux using 4.6 mmol photons/s/m^2 per W/m^2 [15].

2.2. Inoculum and Culture Media

PCC6803 was cultivated in a 5-L mother culture grown in a transparent 10-L reservoir bottle (KIMAX, Germany) to which we sparged filtered air (2 L_{air}/L_{liquid}/min). The bottle was continuously illuminated using fluorescent lamps (20 W/m^2 on the exterior) in the same photo-incubator chamber. Non-limiting N_i, P_i, and other nutrients were supplied using standard BG-11 medium to which we added supplemental P_i using a semi-batch mode of operation (hydraulic retention time $\cong$ 10 d) [16]. Thus, inocula taken from the mother culture had been experiencing feast-and-famine nutrient conditions. Inoculum cultures were kept at a 730-nm optical density (OD_{730}) of 2.3, which is defined as $-\log_{10}(I/I_0)$, where I is the intensity of light at a 730-nm wavelength that has passed through a sample and I_0 is the intensity of the light before it enters the sample.

For the batch experiments, the standard BG-11 growth medium [17] was modified to achieve limitation independently by each nutrient. For all experiments, we removed the usual amount of Na_2CO_3 from standard BG-11 solutions in order to have independent control of C_i; we call this BG-11C, which contained 1.5 g $NaNO_3$, 40 mg $K_2HPO_4{\cdot}3H_2O$, 75 mg $MgSO_4{\cdot}7H_2O$, 36 mg $CaCl_2{\cdot}2H_2O$, 6 mg citric acid, 6 mg ferric ammonium citrate, 1 mg EDTA disodium salt, and 1 ml mixed trace metal solution. Each liter of trace metal solution contained 2.9 g H_3BO_3, 1.8 g $MnCl_2{\cdot}4H_2O$, 0.22 g $ZnSO_4{\cdot}7H_2O$, 0.39 g $NaMoO_4{\cdot}2H_2O$, 79 mg $CuSO_4{\cdot}5H_2O$, and 49 mg $Co(NO_3)_2{\cdot}6H_2O$ in deionized water (conductivity = 18.2 MΩ·cm) produced by Purelab Ultra (ELGA lab water, USA). Further modifications were made to BG-11C for each batch experiments, as described below, and all media were autoclaved before use.

2.3. Batch-experiment Procedures

Table 1 summarizes the four sets of batch experiments to estimate each kinetic parameter. We first used batch experiment 1 to obtain the parameters for LI-controlled kinetics first. It had ample N_i, P_i, and C_i at all times so that only LI was limiting. Using optimal-LI conditions revealed in experiment 1, we then conducted batch experiments 2, 3, and 4 to determine parameters $K_{S,Ni}$, $K_{S,Pi}$, and $K_{S,Ci}$, respectively. The rightmost column in Table 1 indicates the range we tested for each independent variable. In all cases, the stoichiometric consumption of the limiting nutrient was small compared to its starting concentration.

Each series of batch experiment was begun by filling all the test bottles with 97 mL of modified medium according to Table 1 and adjusting the pH to 8±0.2 using a phosphate buffer ($H_2PO_4^-$ and HPO_4^{2-} at a 7:43 mole ratio) and 0.2 M hydrochloric acid or 0.2 M sodium hydroxide. The pH was monitored using a pH meter (Accumet AB15; Fisher scientific, USA) with a glass pH electrode. We put the reactors inside the incubator overnight to establish the same temperature. The next day, we began aeration for the reactors of experiments 1 to 3 using gas-flow-rate controllers (5 L_{air}/L_{liquid}/min) and inoculated 3 mL of mother culture with dis-

Table 1. Experimental Design for the Estimation of Kinetic Constants (The experimental set up for these experiments is shown schematically in Fig. S1 in Supporting Information.)

Experiment Number	Independent Variable (Unit)	Aeration rate (L_{Air}/L_R/min)	CO_2 content (ppmv[a])	C_i supply rate (mgC/L_R/min)	Medium	Test range
1	LI_0 (W/m^2)	5	387	1.04	BG-11C[b]	0 3 5 8 13 18 33 47
2	N_i (mgN/L)	5	387	1.04	BG-11N[c]	0[e] 3 12 49 124
3	P_i (mgP/L)	5	387	1.04	BG-11P[d]	0[e] 1 3 11 27
4	C_i (mgC/L)	0	0	0	BG-11C	0[e] 1 3 14 29 143 286

[a] Part per million by volume from NOAA/ESRL (http://www.esrl.noaa.gov/gmd/ccgg/trends/).
[b] Modified BG-11 without Na_2CO_3.
[c] Modified BG-11C without $NaNO_3$. We independently added $NaNO_3$ to meet the target concentration.
[d] Modified BG-11C without $K_2HPO_4{\cdot}3H_2O$. We independently added $K_2HPO_4{\cdot}3H_2O$ to meet the target concentration.
[e] Control experiment testing zero concentration.

posable syringes and needles. The C_i-supply rate (mg C/$L_{reactor}$ /min) was based on the CO_2-aeration rate (L_{air}/L_R/min), the CO_2 content in the air (~380 ppm, v/v), the ideal-gas assumption (1 mol = 22.4 L) under standard temperature and pressure, and the molar mass of C (12 g/mol). For estimating C_i kinetics in experiment 4, we added different amounts of $NaHCO_3$ to meet the C_i goal of each test, instead of supplying C_i by aeration; this approach allowed us to maintain a constant total-C content (= residual C_i + C in biomass) in each bottle. Although experiment 4 was not aerated, we inoculated it in the usual way and shook the bottles by hand to mix their contents at the start of the experiment and before each sampling. In addition, any produced gas was vented using a disposable syringe needle to relieve pressure build up. Since PCC6803 remained in a stable suspension for up to two days, the liquid contents for experiment 4 experienced no loss of biomass concentration due to sedimentation between samplings. The pH of BG-11C was 8±0.2, which indicates that dominant C_i species was HCO_3^-[18].

The initial OD_{730} ranged from 0.08 - 0.10, which corresponded to 21 - 27 mg/L as dry weight (DW). This low initial biomass concentration and excess concentrations of nutrients guaranteed minimal LI attenuation at the start of the experiment 1. Furthermore, we took samples during the initial 4 hours, when OD_{730} remained less than 0.3 (Fig. S2 in supporting information). Based on our previous research [16], the specific growth rate adjusts to the new experimental conditions for LI, C_i, N_i, and P_i within an hour; thus, the OD_{730} was utilized results from 1 to 4 hours.

We performed quadruplicate experiments in four reactors for each experimental condition. Thus, we present the average specific growth rate with a standard deviation from the 4 reactors. Variability was small among the tests and may have originated from small differences in acclimation to the new conditions, inoculum size, carryover of nutrients with the inoculums, and sampling timing.

2.4. Control Experiments

We also carried out an upper control experiment with all nutrients present and a series of lower control experiments with one nutrient totally absent (Fig. S2 of the Supporting Information). The upper control results indicate that the maximum μ was > 1.6/d. For the lower controls, the inocula were washed three times in 0.9% NaCl solution to remove residual nutrient from the inoculum's growth medium before conducting each experiment. The lower control results document that eliminating any one nutrient suppressed biomass growth so that the biomass increase over 4 h was a maximum of 8.0 mg/L or 0.03 OD_{730}. Because the change in biomass was so small in the lower controls, we were able to use directly the biomass concentrations in non-control experiments (Table 1).

2.5. Sampling and Analytical Methods

The growth kinetics of PCC6803 were monitored by analyzing 2-mL liquid samples taken at 0, 1, and 4 hours with a disposable syringe using a needle inserted through the rubber cap. OD_{730} was directly measured with a UV-visible spectrophotometer (Cary 50 Bio; Varian Inc., USA) at a wavelength of 730 nm and was converted to DW using calibration curve that we determined for PCC6803, shown in Fig. S3 of Supporting Information. For the calibration, DW was determined using total suspended solids (Method 2540D in *Standard Methods*) [19].

The specific growth rate was computed for synthesis based on biomass concentrations in samples taken at the beginning and end of a time interval:

$$\mu = \mu_{syn} - b = \frac{\ln X_4 - \ln X_1}{t_r - t_1} \tag{2}$$

where μ is the observed net specific growth rate (/d), μ_{syn} is the synthesis specific growth rate (/d), b is the endogenous decay rate (/d), and X_1 and X_4 are the biomass concentrations (expressed by mg DW/L) at time 1 and 4 hours (t1 and t4), respectively. Independently, b was estimated by the decrease of biomass concentration under complete darkness for one day; the value was 0.056/d, as presented in Table S1 of Supporting Information.

To obtain the best estimates of the kinetic parameters for LI, N_i, P_i and C_i from each set of experiments, we conducted non-linear regression using computer software (Sigmaplot10; Systat Software Inc., USA) that related the observed μ_{syn} = m + b to the spatially averaged light intensity (LI_{SA}) and the concentration of the limiting nutrient at a fixed LI_{SA}. For estimating parameters associated with N_i, P_i, and C_i kinetics in experiments 2 - 4, we employed the best-fit constants for LI revealed previously in experiment 1. This strategy made it possible for us to use the experimental data for each nutrient to estimate only one corresponding parameter (K_S).

2.6. Light Attenuation

Average LI integrated over the entire PBR volume representing spatially averaged value, LI_{SA}, was computed in W/m^2 as PAR based on these assumptions: a transparent cylindrical shape, uniform illumination from all sides, no light absorption by the biomass-free medium, no top and bottom effects, and light attenuation according to the Beer-Lambert law [12]. Supporting Information (Fig. S4) provides details of the geometry involved in the calculation. The equation for LI_{SA} is:

$$\begin{aligned} LI_{SA} &= \frac{LI_o}{A_n} \int_0^{2\pi} \int_0^R I_n(X, r, \theta) \cdot r \, dr \, d\theta \\ &= \frac{LI_o}{\pi R^2} \int_0^{2\pi} \int_0^R e^{-\epsilon X [r \cdot \cos\theta + \sqrt{R^2 - r^2 \cdot \sin^2\theta}]} \, r \, dr \, d\theta \end{aligned} \tag{3}$$

where LI_0 = LI at the light-entry surface (W/m^2), ε = the Beer-Lambert constant (m^3/g/m), X = biomass concentration (g/m^3), and d = depth of light path from the light-entry surface (m). Suh and Lee[11] verified that e for *Synechococcus* sp. PCC6301 is 0.255 m^3/g/m. Our preliminary study with PCC6803 found a similar value from light-attenuation experiments conducted within our ranges of biomass concentration using PCC6803. Therefore, we utilized ε= 0.255 m^3/g/m to calculate LI_{SA}, and the LI_{SA} was used in Eq. (1).

3. Results and Discussion

3.1. Substrate-Inhibition Kinetics for LI_{SA}

Table S2 shows the calculated LI_{SA} values using the measured biomass concentrations and LI_0 at the start of each batch

experiment. With LI_0 outside the batch reactor varying from 2.6 to 48 W/m^2, LI_{SA} ranged from 2.3 to 43 W/m^2. The attenuation was ~13% of LI_0 with the biomass concentrations of ~ 27 mg DW/L.

Fig. 1 shows the observed μ_{syn} values based on biomass measurements taken at 1 and 4 hours for each LI_{SA}. These results demonstrate the expected trends for LI self-inhibition kinetics: increasing μ_{syn} for LI_{SA} up to about 8 W/m^2, relatively steady μ_{syn} from about 10 to 25 W/m^2, and decreasing μ_{syn} for LI_{SA} greater than ~30 W/m^2. The highest observed μ_{syn} was approximately 1.5/d for LI_{SA} of ~30 W/m^2.

Non-linear regression yielded μ_{max} of 2.8/d, K_L = 11 W/m^2, and $K_{L,I}$ = 39 W/m^2. The value of μ_{max} was significantly larger than 1.5/d because of LI inhibition, which is quantified by the relatively low value of $K_{L,I}$. Based on the LI kinetic model, PCC6803 achieve a benchmark $\mu_{syn} \geq$ 1/d when LI_{SA} is in the range of 7 to 62 W/m^2.

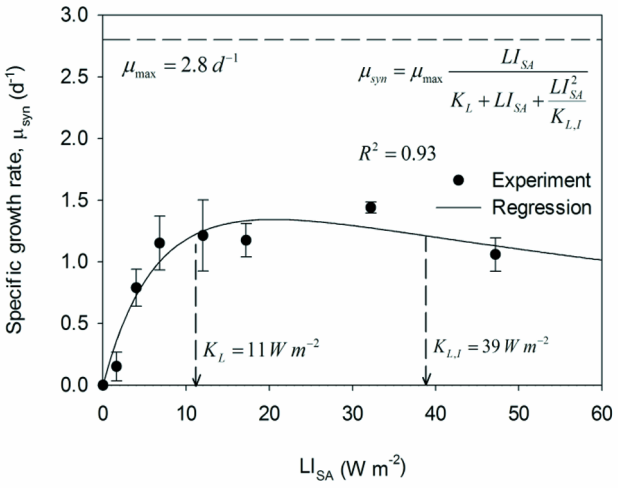

Fig. 1. Specific growth rates (μ_{syn}) for the range of LI_{SA} values for experiment 1 and the corresponding regression results using the self-inhibition kinetic model (inset).

3.2. Monod Kinetics for N_i, P_i, and C_i

Using the results for LI kinetics (experiment 1, which had sufficient N_i, P_i, and C_i), we could obtain parameter estimates for the growth kinetics of PCC6803 for each nutrient limitation (experiments 2 – 4). We chose LI_0 = 18 W/m^2 to keep the initial LI_{SA} at around 16 W/m^2, which is in the range where LI_{SA} does not have a sensitive effect on μ_{syn} (Fig. 1). In each experimental series, we computed the LI-adjusted μ_{max} value from Eq. (1) and the actual LI_{SA} value; we call this value μ_{syn}, LI.

Experiment 2 evaluated the kinetics for N_i limitation, and Fig. 2 shows μ_{syn} for N_i varying from 0 to 124 mgN/L. The results clearly exhibit a Monod-type saturation pattern with μ_{syn} saturating at $\mu_{syn,LI}$ = 1.6/d (based on LI limitation from experiment 1). Non-linear regression (R^2 = 0.97) yields $K_{S,Ni}$ = 1.4 mgN/L. This value means that the NO_3^- concentration in a PBR should be greater than 13 mgN/L to maintain 90% of the maximum specific growth rate, but the rate is slowed to 10% of the maximum value when the NO_3^- concentration falls below 0.16 mgN/L. Serious N_i-limitation of cyanobacteria is highly undesirable, because it can cause visible chlorosis, or yellowish coloring of the cells due to the degradation of thylakoid membranes [20] or a decrease in their chlorophyll content [21]. Keeping N_i over 2.3 mgN/L in a PBR is necessary for growing PCC6803 at a benchmark μ_{syn} of at least 1/d (with LI_{SA}~16 W/m^2).

Fig. 3 shows the photosynthetic specific growth rate as a function of the total-P_i concentration in experiment 3. With the pH held constant at 8±0.2, the dominant P_i species were $H_2PO_4^-$ and HPO_4^{2-}, present at a molar ratio of 7:43. Thus, the corresponding ranges for $H_2PO_4^-$ and HPO_4^{2-} concentrations were 0.14-3.8 mgP/L and 0.86-23 mgP/L, respectively. The experiments showed an almost constant μ_{syn} value (1.6/d) for total-P_i concentrations of 3 mgP/L and higher. Only when the total-P_i concentration was less than 1 mgP/L, the lowest concentration tested except the control, did μ_{syn} show a slightly lower value, 1.4/d.

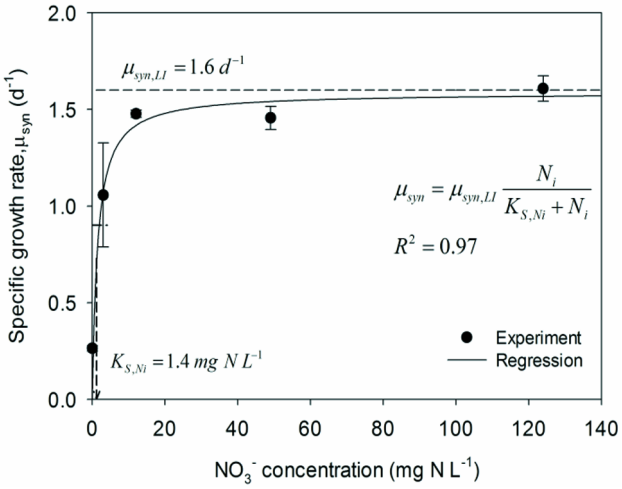

Fig. 2. Specific growth rates (μ_{syn}) for the range of N_i concentrations for experiment 2 and the corresponding regression results using the Monod model (inset).

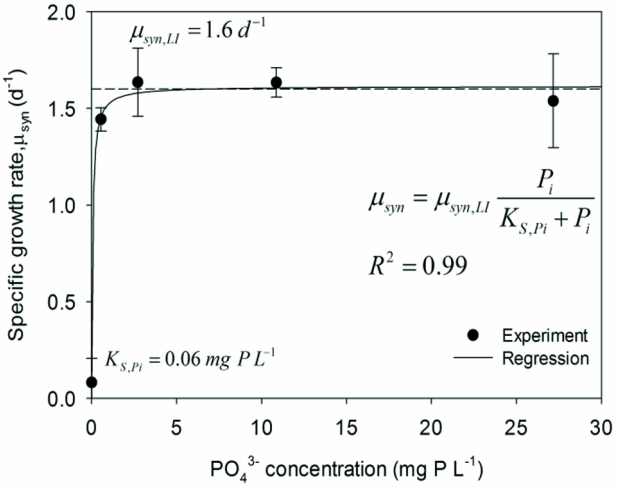

Fig. 3. Specific growth rates (μ_{syn}) for the range of P_i concentrations for experiment 3 and the corresponding regression results using a Monod model (inset). At pH 8, the molar ratio of $H_2PO_4^-$: HPO_4^{2-} = 7:43, while H_3PO_4 and PO_4^{3-} are negligible.

Applying $\mu_{syn,LI}$ (1.6/d) gave a good match of the Monod function to the experimental results and led to an estimated $K_{S,Pi}$ of 0.06 mgP/L for total P_i (R^2 = 0.99). This low $K_{S,Pi}$ value indicates very high affinity for P_i and that it is not necessary to maintain a high concentration of P_i in the PBR, compared to what is required for N_i. For example, maintaining $\mu_{syn} \geq$ 1/d demands that the total-P_i concentration be at least 0.1 mgP/L.

Although a high specific growth rate does not require a high P_i concentration, it is very important to monitor the P_i concentration and ensure that it does not become seriously limiting. Previous studies reported that a deficiency of P_i may have an important impact on photosynthetic activity, since P is a key component in energy transfer and signal transduction, as well as lipid biosynthesis for algae and cyanobacteria [22]. Once P_i-limitation sets in, it may cause severe change of photosynthesis in PCC6803, as has been noticed for other cyanobacteria [23]. Problems of P_i depletion can be exacerbated when complexation of HPO_4^{2-} and PO_4^{3-} with cations such as Ca^{2+} and Mg^{2+} reduces the availability of P_i by forming octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$) and hydroxyapatite ($Ca_{10}(OH)_2(PO_4)_6$), particularly as the pH increases [24].

In experiment 4, we varied the C_i concentration using $NaHCO_3$ as the C_i source, not by aeration. This ensured that our results were not confounded by mass transfer resistance from $CO_{2(g)}$ to $CO_{2(aq)}$. The pH was maintained at 8 with a phosphate buffer; thus, the mass distribution between $CO_{2(aq)}$ and HCO_3^-, the C_i sources for PCC6803 [25], was fixed at a molar ratio of 1:49. The corresponding test ranges for $CO_{2(aq)}$ and HCO3- were 0-5.6 mgC/L and 0-279 mgC/L, respectively.

Fig. 4 illustrates a clear Monod-like relationship between μ_{syn} and the C_i concentration. The experimentally observed value peaked at around 1.5/d for C_i. Setting the maximum specific growth rate as $\mu_{syn,LI}$ = 1.6/d (from LI_{SA} and experiment 1) gave an excellent match with the experimental results, and nonlinear regression estimated K_S, C_i = 0.6 mgC/L for total C_i. Thus, PCC6803 had a high affinity for C_i when HCO_3^- was the dominant species at pH around 8. Due to its large dominance over $CO_{2(aq)}$ in solution, HCO_3^- probably was the species taken up by PCC6803 in these experiments [26], and $K_{S,Ci,}$ = 0.6 mgC/L probably corresponds to the K_S value for HCO_3^-. The C_i concentration needed to achieve μ_{syn} of 1/d is approximately 1.0 mgC/L, which is intermediate between 0.1 mgP/L and 2.3 mgN/L.

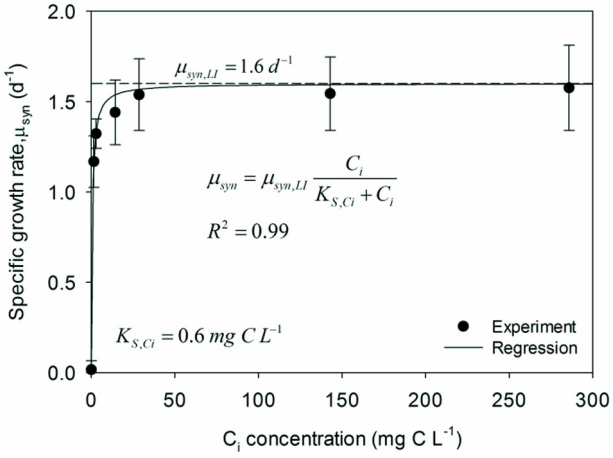

Fig. 4. Specific growth rates (μ_{syn}) for the range of C_i concentrations for experiment 4 and the corresponding regression results using a Monod model (inset). At pH 8, the molar ratio of $CO_{2(aq)}$: HCO_3^- : CO_3^{2-} = 1.9 : 97.6 : 0.5.

3.3. Interpretation of Kinetic Parameters for the Nutrients

Table 2 compares our LI parameters to literature values obtained for other cyanobacteria and algae. Our K_L value was similar to other results (6 - 18 W/m^2), but $K_{L,I}$ was considerably smaller than previously reported values. Most notably, *Synechococcus* sp. PCC7942 had a reported $K_{L,I}$ of 97 W/m^2 [27], although light attenuation was not considered in the estimation. Using LI_{SA} instead of LI0 would make their $K_{L,I}$ smaller: ~77 W/m^2 based on the analysis presented in Supporting Information (Table S3 and Fig. S5). Thus, the comparison of $K_{L,I}$ values suggests that PCC6803 is relatively more sensitive to photo-inhibition than are other phototrophs that have been evaluated. However, significant negative impacts of LI self-inhibition require a substantially greater LI_{SA} value than ~62 W/m^2 (Fig. 1).

Table 3 also shows all of our K_S and μ_{max} values together with literature values obtained with other algae or cyanobacteria. Compared to ranges for algal species with reported μ_{max} values (0.7 – 1.7/d), PCC6803 had by far the highest μ_{max} (2.8/d), although the highest observed $\mu_{syn,LI}$ was 1.6/d due to photo-inhibition. The relatively high μ_{max} values suggests that PCC6803 behaves as an r-strategist, or a copiotroph that employs a rapid-growth strategy

Table 2. Comparisons of Kinetic Parameters using the Self-inhibition Model for Light Irradiance for Cyanobacteria and Algae

Photosynthetic Microorganisms	Dependent Parameter	K_L (W/m^2)	$K_{L,I}$ (W/m^2)	Reference
Synechococcus sp. PCC7942	Photosynthetic production of Ethylene	5.8 b	97 b	[27]
Spirulina platensis	Specific growth rate	10	180	[32]
Anabaena flos-aquae	Specific growth rate	14	61	[33]
Synechocystis sp. PCC6803	Specific growth rate	11	39	This study
Chlamydomonas reinhardtii[a]	Specific growth rate[c]	18	540	[34]
Chlorella vulgaris[a]	Photosynthetic activity	11 d	-	[35]

[a] Algae

[b] The unit of lumen m^{-2} (lx) was converted to W/m^2 by assuming the luminous efficacy of radiation as natural sunlight (93 lumen/W). Supporting Information shows the result of LI-adjustment to LI_{SA} as PAR

[c] Estimated by using a continuous reactor

[d] The unit of mE/m^2/s was converted to W/m^2 by assuming 1 W/m^2 as 4.6 mE photon/m^2/s

Table 3. Comparisons of μ_{max} and K_S Values for C_i, N_i, and P_i using Monod Kinetics with a Range of Phototrophic Microorganisms

Photosynthetic organisms	Target nutrient	μ_{max} (d^{-1})	K_S (mg/L)	Reference
Chlorella[a]	C_i	1.7	0.003	[36]
Anabaena flos aquae[b]		1.3	0.002	
Oscillitoria[b]		0.7	0.005	
Synechocystis sp. PCC6803[b]		2.8	0.5	This study
Chlorella vulgaris[a]	N_i	0.2	32	[31]c
Spirulina platensis 8005[b]		-	5.3	[9]
Ankistrodesmus falcatus[a]		1.2	0.13	[37]
Asterionella Formosa[a]		1.0	0.02	
Fragilaria crotonensis[a]		0.9	0.11	
Scenedesmus sp.[a]		1.4	0.16	
Synechocystis sp. PCC6803[b]		2.8	1.4	This study
Chlorella vulgaris[a]	P_i	0.1	10.5	[31] c
Anabaena flos-aquae[b]		0.9	0.04	[38]
Ankistrodesmus falcatus[a]		1.1	0.12	
Asterionella Formosa[a]		1.0	0.02	
Fragilaria crotonensis[a]		0.9	0.03	
Microcystis sp.[b]		1.2	0.05	
Scenedesmus sp.[a]		1.4	0.02	
Synechococcus PCC7942[b]		-	0.07	[30]
Cylindrospermopsis raciborskii[b]		1.0	0.002	[39]
Synechocystis sp. PCC6803[b]		2.8	0.06	This study

[a] algae; [b] cyanobacteria; [c] NH_4^+ was used as a nitrogen source to test wastewater treatment.

[28] that gives it a competitive advantage when conditions allow it to avoid significant limitation by LI or any of the inorganic nutrients.

The values of $K_{S,Ci,}$ reported for algae (0.002-0.005 mgC/L) are significantly lower than for PCC6803. This means that the growth rate of PCC6803 can be more easily limited by C_i, compared to algae, when an imbalance between supply and utilization of CO_2 occurs. Thus, PCC6803 needs to have a sufficient C_i supply rate to avoid HCO_3^- limitation, which would counteract it growth-rate advantage over algae.

Due to PCC6803's relative sensitivity to the C_i concentration, it would be possible that high pH may slow the C_i-uptake kinetics by changing the dominant C_i form to CO_3^{2-}, with CO_2(aq) becoming negligible because the concentrations of available C_i species - $CO_{2(aq)}$ and HCO_3^- - are determined by the pH [25]. The pH also affects the complexation equilibria of cationic and anionic species that can precipitate with C_i. High pH by active photosynthesis can lower the available C_i by precipitating it as part of $CaCO_3$.

Similar to C_i, PCC6803 is more easily limited by low N_i, compared to other phototrophs for which we have reports [16, 29]. Although our estimated $K_{S,Ni}$ (1.4 mgN/d) is not far from a literature value (5.3 mgN/L) obtained for *Spirulina platensis* [9], which, like *Synechocystis*, is a non-diazotrophic cyanobacterium [20], several algae show $K_{S,Ni}$ values about one order of magnitude lower (0.02~0.16 mgN/L).

Unlike for C_i and N_i, PCC6803 is not more severely limited by P_i than are other phototrophs. A study using the cyanobacterium *Synechococcus* sp. PCC7942 reported $K_{S,Pi}$ = 0.07 mgP/L [30], almost identical with our result (0.06 mgP/L). In addition, the $K_{S,Pi}$ values of several algae were in a similar range, 0.02 to 0.12 mgP/L, although one report was much higher [31]. Such similarity may indicate that utilization mechanisms of P_i are invariant.

4. Conclusions

We use a series of special batch experiments to estimate parameter values for a multi-component kinetic model of photoautotrophic growth. Results for the cyanobacterium *Synechocystis* PCC6803 quantify its growth-rate limitation by LI_{SA} or the concentration of C_i, N_i, or P_i. With μ_{max} = 2.8/d, PCC6803 is an r-strategist (or copiotroph) that can grow fast when light and nutrients do not limit. This is ideal when the goal is to maximize the biomass production rate, such as for biomass production as a renewable source of biofuels and biomaterials. To gain the advantages of its high maximum specific growth rate, PCC6803 needs to have moderate light illumination (7 – 62 W/m^2 for $\mu_{syn} \geq$ 1/d) and relatively high nutrient concentrations: $N_i \geq$ 2.3 mgN/L, $P_i \geq$ 0.1 mgP/L, and $C_i \geq$ 1.0 mgC/L.

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Supplementary Information

Supplementary data to this article can be found online at http://eeer.org.

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