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Short communication

Light attenuation changes with photo-acclimation in a culture of *Synechocystis* sp. PCC 6803☆

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ABSTRACT

An inherent complication in the relationship between light intensity (LI) and microalgae growth rate is that light attenuates through a culture due to its absorption by biomass. While a biomass's specific extinction coefficient (ε describing how rapidly light attenuates) often is assumed to be a constant for a species for mathematical modeling, it is well documented that pigmentation and light absorption depend on growth conditions, particularly light intensity itself. In this study using *Synechocystis* sp. PCC 6803, we investigated the effect of LI on ε. Using cultures fully acclimated to the LI at which they were grown, we found that biomass grown at higher LI absorbed less light than biomass grown at lower LI; thus, ε was larger for lower LI. We quantify the relationship between ε and the acclimated LI and suggest that ε would be an appropriate metric for describing photo-acclimation.

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1. Introduction

Mathematical modeling of microalgal growth is a valuable tool for optimizing biomass productivity in engineered systems and understanding primary production in natural systems [1]. A growth-limiting factor that has received much attention is light, because its intensity varies naturally, and light is the energy source driving photosynthesis [2]. Unlike a growth-limiting nutrient, light attenuates through a microalgal culture, which means that the light intensity (LI) declines away from its source. Understanding light attenuation is important, because it controls the LI available to cells within the culture and because light attenuation is partly caused by light-energy absorption of the biomass [3,4].

Light attenuation is most often represented mathematically by the Beer-Lambert equation, which utilizes an exponential function based on the light path (d, in m), biomass concentration (X, in $mg \cdot L^{-1}$), and an extinction coefficient (ε, in $m^2 \cdot g^{-1}$) to represent the light absorption of the microalgae culture [2,5]:

$$LI = LI_0 \exp(-\varepsilon X d) \quad (1)$$

where LI is the local light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$), and LI_0 is the incident light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$). In practice, ε is empirically determined and accounts for the aggregate effect of light scattering and light absorption [4]. While in-depth analyses of the radiative properties of microalgae suggest that scattering is an important phenomenon in microalgae LI extinction, they also report that the vast majority (about 99.9%) of scattering occurs in the forward direction [6–9]. Forward-scattered light can be absorbed by other biomass; thus, only back-scattered light at the light's entering surface and light scattered out of the edges of the reactor are truly lost to scattering.

Light absorption depends on the pigmentation of the biomass, while light scattering depends on cell morphology and surface properties [10]. It is well established that microalgae change their pigmentation and cell morphology in response to different light conditions as a component of photo-acclimation [10–12]. Microalgae also change their pigmentation in response to adverse growth conditions, such as nutrient limitation, non-optimal salinity, or extreme LI [13]. However, the common practice in modeling light attenuation is to assume a constant ε for a given species of microalgae [2,14–17]. Although the phenomena of changing ε with growth conditions has not received much attention, past research with *Chlorella vulgaris* demonstrated that ε depended on culture biomass density [5,18].

A metric that has been used to identify the photo-acclimation state in microalgae growth models is the ratio of chlorophyll-to-carbon [11,19,20]. For most species, however, the biomass contains significant amounts of non-chlorophyll pigments, and the primary pigments in cyanobacteria are phycobilisomes, not chlorophylls [11,21,22]. For these reasons, chlorophyll content is not a good metric for absorbance

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or photo-acclimation. Alternatively, we suggest that ε is a better metric than the chlorophyll-to-carbon ratio for identifying the photo-acclimation state, as it accounts for the aggregate effect of all pigment and morphological changes.

In this study, we use the cyanobacterium *Synechocystis* sp. PCC6803 (simply *Synechocystis* from here) to test the hypothesis that the LI to which microalgae are acclimated systematically affects its ability to absorb light. In particular, photo-acclimation affects ε such that biomass grown at low LI has a higher ε than biomass grown at high LI. This finding also suggests that ε can be an appropriate parameter to represent photo-acclimation.

2. Materials and methods

Synechocystis was grown in a Photobioreactor FMT150 (Photon Systems Instruments, Drásov, Czech Republic; simply FMT from here) with nominal incident light settings from 0 to 6626 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of Photosynthetically Active Radiation (PAR), a liquid volume of 370 mL, and a fixed temperature of 30 °C. The FMT is described in detail by Nedbal et al. (2008) [23]. We replaced the factory-supplied diffusor with an Aquarium Fine Bubble Air Stone (Top Fin®, Phoenix, AZ), and air was supplied by an EcoPlus® aquarium air pump at approximately 0.1 $L \cdot min^{-1}$ (Sunlight Supply, Inc., Vancouver, WA) and filtered through a 0.2-μm membrane bacterial air vent (Pall Corporation, Ann Arbor, MI). The FMT took automatic readings of optical density at 735 nm (OD_{735}) and 680 nm (OD_{680}). The operating conditions utilized the Turbidostat Module, which added fresh growth medium using a peristaltic pump when the OD_{735} reached an upper set value, and it stopped delivering medium when it reached a lower set value. We set the OD_{735} range at 0.20 to 0.21. The pH was controlled using an MC122 pH Controller (Milwaukee Instruments, Rocky Mount, NC), which opened a solenoid valve (Milwaukee Instruments, Rocky Mount, NC) to bubble pure CO_2 into the reactor when the pH exceeded 8.5 maintaining a pH between 7.5 and 8.6. Growth medium was autoclaved standard BG-11 as described by Rippka et al. [24]. The FMT cultivation vessel was autoclaved and inoculated from a flask seed culture.

The FMT had nominal light settings ranging from 0 to 6626 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. To determine the actual incident light intensities, we used a LI-190 PAR sensor (LI-COR Biosciences, Lincoln, NE) and measured the light entering the cultivation vessel directly behind a piece of glass placed where the inside wall of the FMT cultivation vessel would be. We measured 9 positions equally spaced over the irradiated area (Fig. S1 in Supplemental Information) and at 23 different nominal light settings ranging from 0 to 3200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. The LI was not uniform, with higher light intensity in the center and less intensity towards the top and bottom of the vessel (Fig. S2). We computed an area-weighted average of the nine points to provide an average incident light reading at each of the light settings tested. The result was a calibration between the nominal FMT light setting (LI_{FMT}) to the actual average incident light intensity (LI_0): $LI_0 = 2.06 * (LI_{FMT} - 81.2)^{0.826}$ (standard error = 8.86 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Because the biomass concentration was relatively dilute and the OD_{735} range within the FMT narrow, we used the average LI (LI_{ave}) as an approximation of the photo-acclimated LI (LI_{acc}) of the culture. LI_{ave} was computed as an area integration of the Beer-Lambert equation:

$$LI_{ave} = \frac{LI_0(1 - \exp(-\varepsilon X w))}{\varepsilon X w} \quad (2)$$

where w is the width of the bioreactor (0.024 m for the FMT).

We independently determined ε by taking a 20-mL culture sample and placing it in a 60-mm × 15-mm petri dish (VWR®, Radnor, PA) with a liquid depth of 9 mm. The sample was illuminated from underneath with a 54-W fluorescent lamp (Hydrofarm, Inc., Petaluma, CA), and the light intensity was measured above the sample using the LI-190 PAR sensor. The sample was then diluted and measured again. Once 5 different dilutions (100% sample, 80%, 60%, 40%, and 20%) and a water control were measured, the data were used to determine ε of the sample by fitting the X and LI data to the Beer-Lambert Equation (Eq. (1)), where d was 0.009 m, and LI_0 was approximately 320 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (the reading for water) (Fig. S3). Dry weight (X) was measured by taking a 0.7-μm glass microfiber filter (Whatman®, Buckinghamshire, UK) and drying it overnight at 60 °C, weighing it, filtering 10 mL of culture through it, drying it at 60 °C overnight again, and subtracting the mass before from the mass after. During this study the biomass dry weight concentration was between 94 and 177 $mg \cdot L^{-1}$.

All ε values are reported for steady-state operating conditions, which we determined after OD_{680}/OD_{735} and growth (as determined by time between dilutions) were stable. Due to turbidostat operation, the dilution rate was tied to growth rate, which was between 1.5 and 2.5 d^{-1} for $LI_{ave} > 125$ $\mu mol \cdot m^{-2} \cdot s^{-1}$ and as low as 0.25 d^{-1} at $LI_{ave} = 13$ $\mu mol \cdot m^{-2} \cdot s^{-1}$. Light acclimation, however, was independent of dilution rate. When the previous LI was lower than the LI being considered, steady-state typically was achieved 2 to 3 days after changing the light, and it took about 1 day when the previous LI was higher. Each measured ε, along with the corresponding X and LI_0, was used to compute LI_{ave} for that point using Eq. (2).

The reactor vessel periodically had visible biofilm and floc formation, particularly at higher LI_{ave}. When this occurred, we removed the culture from the reactor vessel, scrubbed the vessel with bleach and Alconox® cleaner (Alconox, Inc., White Plains, NY), rinsed it thoroughly, filtered the culture through sterile cheese cloth, and returned the culture to the vessel. We discarded all data collected when the FMT contained visible biofilms or flocculated biomass.

All curve fittings, including the ε determinations described above, and all best-fit parameters in Eq. (3) (below) were obtained by least-squares fitting between the experimental and modeled results, and standard errors were calculated [25]. The plot of residuals was generated by subtracting ε predicted from Eq. (3) from the measured ε.

3. Results and discussion

Measured ε values for LI_{ave} (assumed to be equal to the photo-acclimated LI; LI_{acc}) with dilute biomass concentrations are displayed in Fig. 1, which clearly shows that ε was not constant. Instead, ε declined from its maximum (ε_{max} = ~0.18 $m^2 \cdot g^{-1}$) at very low LI_{acc} and stabilized at a minimum level of approximately 0.045 $m^2 \cdot g^{-1}$ (ε_{min}) as LI_{acc} becomes very large. We mathematically represent the systematic changes in ε using:

$$\varepsilon = \frac{(\varepsilon_{max} - \varepsilon_{min}) k_\varepsilon}{k_\varepsilon + LI_{acc}} + \varepsilon_{min} \quad (3)$$

where k_ε is the half maximum light absorption LI_{acc} ($\mu mol \cdot m^{-2} \cdot s^{-1}$), ε_{max} is the measured ε value at the smallest LI_{acc} able to sustain net positive growth, and ε_{min} is extrapolated from the ε trend as LI_{acc} approaches infinity. The best-fit values for the experimental data are summarized in Table 1 and were used to produce the model line in Fig. 1.

The value of ε was most sensitive to LI_{acc} in the region of lower LI_{acc}, where ε increased steadily as LI_{acc} declined. The higher ε at low LI_{acc} also was qualitatively apparent by the culture appearing greener than cultures grown at higher LI. This trend supports that *Synechocystis* maximized light absorption when light was scarce by increasing light-absorbing pigments. At the other end of the LI_{acc} range, the ε value changed proportionally less as LI_{acc} increased to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This trend is similar to chlorophyll measurements taken for *Chlorella* [11,19] and for *Synechocystis* [26] (although quantitatively quite different for *Synechocystis*, as chlorophyll is only one component of photo-acclimation). The residuals plot of Fig. 1 demonstrates that variability of the measured ε was random throughout the range of LI_{acc} tested, although the magnitude of the variability was slightly larger for lower

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