

Overyielding potential of microalgal polyculture with complementary light absorption spectra: a model-based analysis

Sihao Di, Aidong Yang*

Department of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PJ, UK

*Corresponding author. Email: aidong.yang@eng.ox.ac.uk

Abstract

Several recent experiments of microalgal polyculture have observed an increased yield compared to monocultures, leading to the suggestion that improved use of the available light resource due to complementary light absorption spectra may be among the underlying reasons. Using numerical simulation, this work explores the impact of conditions (including both operation settings and species traits) on the possibility of biomass overyielding by a co-culture of two algal species. From the study of a system with simplified light absorption spectra, it was found that both operation settings such as incident light intensity and loss rate of algal biomass and species traits such as light use efficiency and maximum specific growth rate can affect the possibility and degree of overyielding. Co-culture of two actual species with light absorption spectra that exhibit complementarity was also simulated, which further confirmed the importance of some of these factors in a realistic case. This work thus suggests that complementary light absorption alone may lead to overyielding in a polyculture of microalgae, only when a number of biological and operational conditions are met.

Key words: microalgae, polyculture, complementarity, overyielding, light absorption spectra, co-existence

1. Introduction

In the last few decades, microalgae have been considered to possess great potential for a variety of industrial applications. A lot of existing work has focused on developing practical algal biotechnology for biofuel production [1-3]. Microalgae-based systems have also frequently been considered for biological wastewater treatment [4] [5] and CO₂ sequestration [6, 7]. Microalgae can be cultured by

either a mono-culture system, or a poly-culture system that grows multiple micro-algal species. Some of poly-culture systems aim at overyielding, which means the collective yield of multiple microalgae species, in terms of either whole biomass or a specific biomass component such as lipids, exceeds that of any single species when cultivated under the same condition [8-11, 12, 13]. A key mechanism that has been used to explain overyielding in a poly-culture system is the complementarity effect [8] [9]. Complementarity refers to resource partitioning or facilitation among different species causing a more complete consumption of the involved resources, which may lead to an increase in the overall yield. In particular, the complementary use of light at different wavelengths by different algal species has been speculated by several authors as a possible explanation of experimentally observed overyielding in poly-culture systems (e.g. [8], [9], [14], [15]) which, if confirmed, can be taken as a guide principles for designing productive co-culture systems. However, this hypothesis is yet to be evaluated.

This work attempts to evaluate the above hypothesis, by means of modelling. In the past, several model-based analyses have been made on the co-growth of multiple phytoplankton species in connection of light utilisation. The rather early work in [16] confirmed that a growth rate model that takes into account the effect of the spectral qualities of light could predict the co-existence of multiple photosynthetic microorganisms in a well-mixed environment. The more recent work in [17] and [18] showed that red and green picocyanobacteria, using the pigments phycoerythrin and phycocyanin to absorb green and red light respectively, could co-exist due to the complementarity in light use, as confirmed by experimental observations. Theoretical analysis was also carried out on multiple species competing over light (as a homogeneous resource) and nutrients, which revealed conditions for co-existence in such circumstances [19] [20]. It should be noted that the above model-based or theoretical work has all focused on co-existence of multiple algal species, but not on overyielding which is a key objective of the engineering systems based on algal co-cultures.

Focusing on overyielding, this work aims to theoretically examine the hypothesis that complementary light utilisation leads to overyielding in microalgal poly-culture, as proposed in some previous work. A mathematical model is formulated to predict the potential of overyielding as a function of several influencing factors. The model is then used in the numerical simulation of a two-species system, with

hypothetical (simplified) and actual light absorption spectra, to explore the conditions for overyielding to occur in a poly-culture system. To our knowledge, this is the first attempt to study the relationship between (i) complementary light absorption between different algal species to be co-cultured and (ii) the overyielding potential of such systems. The findings from this study have the potential to provide insight to the design of algal co-culture systems.

2. Methodology

2.1 The modelling approach

This work considers light supply as the only limiting factor to the growth of microalgae; nutrients such as nitrogen and phosphorus are assumed sufficient for cultivation. Mass balance of microalgae species i in a well-mixed bioreactor follows equation (1):

$$\frac{dN_i}{dt} = \frac{N_i p_{i_{max}}}{z_m} \int_0^{z_m} f_{I_i}(z) dz - L_i N_i \quad (1)$$

where N_i represents the cell density (i.e. number of cells per unit reactor volume), $p_{i_{max}}$ is the maximum specific growth rate, z is the vertical position in the bioreactor between the top ($z = 0$) and the bottom ($z = z_m$) surface, L_i is the rate of biomass loss, assumed to be dominated by the dilution rate in a continuous bioreactor, a parameter that can easily be manipulated. In this work, the loss rate is an important parameter, as it directly affects the steady-state concentration of cells which in turn influences the extent of light utilisation by the algal culture. The light factor f_{I_i} indicates the extent to which the algal growth is affected by light.

Light attenuation is modelled by applying the Beer-Lambert law:

$$I(\lambda, z) = I_{in}(\lambda) \exp(-\sum_{i=1}^n k_i(\lambda) N_i z) \quad (2)$$

$I(\lambda, z)$ represents the light intensity (measured in number of photons per unit area per unit time) of wavelength λ at depth z , $I_{in}(\lambda)$ is the incident light intensity of wavelength λ , $k_i(\lambda)$ is the specific light absorption spectrum of species i . The amount of light absorbed by species i at depth z over the photosynthetic active radiation (PAR) wavelength range of 400-700 nm, denoted as $\gamma_i(z)$, is

$$\gamma_i(z) = \int_{400}^{700} I(\lambda, z) k_i(\lambda) d\lambda \quad (3)$$

$\gamma_i(z)$ is light absorbed by species i at depth z , on a per cell basis.

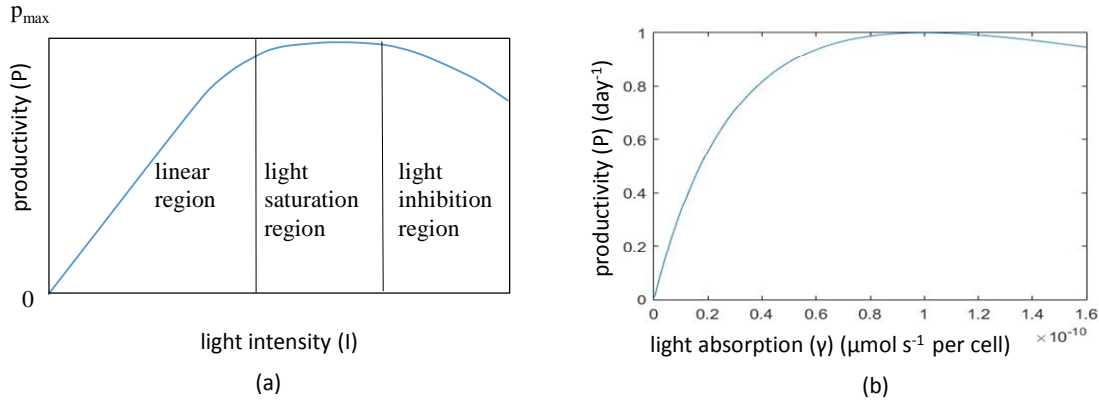


Figure 1. Relationship between photosynthesis and light supply or absorption. (a) Typical relationship between photosynthetic productivity and light intensity (“P-I curve”); (b) Predicted photosynthesis rate as a function of light absorption (“P- γ curve”) according to equation (4b) and using parameters in Table 1.

To quantify the light factor f_{I_i} introduced in equation (1), the typical relationship between photosynthetic productivity for microalgae (denoted by ‘ P ’) and light intensity (denoted by ‘ I ’) is considered, as shown on Figure 1a. Three regimes are distinguished according to the level of light intensity [21].

- At low light intensity levels, the productivity is proportional to light intensity, the limiting step in this regime is the capture of photons by microalgae.
- As the level of light intensity increases, the rate of photosynthetic reactions becomes slower than the rate of photon absorption by microalgae, and hence approaches saturation.
- If the light intensity increases further and beyond an inhibition threshold, damage of key proteins in the photosynthetic units occurs and consequently the productivity drops [21].

The above P - I curve is widely adopted when no details of light absorption spectrum are considered. In this work, it is adapted by replacing light intensity (I) with light absorption (γ) to allow the photosynthesis rate to be directly related to the actual amount of absorbed light, which is similar to the modelling approach in a previous work [17]. Therefore, the above P - I curve essentially becomes a P - γ

curve (Figure 1b). Quantitatively, the light factor in equation (1) is modelled as a nonlinear function of light absorption, reflecting the three regimes mentioned above and adapting a model previously formulated for the $P-I$ curve [22]:

$$f_{I_i}(z) = \frac{\gamma_i(z)}{a_i \gamma_i(z)^2 + b_i \gamma_i(z) + c_i} \quad (4a)$$

$$P_i(z) \equiv P_{i_{max}} f_{I_i}(z) = P_{i_{max}} \frac{\gamma_i(z)}{a_i \gamma_i(z)^2 + b_i \gamma_i(z) + c_i} \quad (4b)$$

The three parameters are calculated by $a_i = \frac{p_{i_{max}}}{\gamma_{i_{opt}}^2 \phi_i}$, $b_i = 1 - \frac{2p_{i_{max}}}{\phi_i \gamma_{i_{opt}}}$, $c_i = \frac{p_{i_{max}}}{\phi_i}$, ϕ_i is the light use efficiency, which, as pointed out in [22], corresponds to the initial slope of the $P-\gamma$ curve defined by Equation 4b. $\gamma_{i_{opt}}$ is the optimum level of light absorption corresponding to the maximum photosynthetic productivity.

2.2 Investigating a two-species community with simplified light absorption spectra

To theoretically assess the biomass overyielding potential due to complementary light absorption, it is desirable to obtain easy control over the degree of complementarity between different species. To this end, a hypothetic community of two species with a pair of simplified light absorption spectra was considered. Each spectrum comprises two parts of equivalent size in terms of the PAR wavelength range, i.e. 400-550 nm and 550-700 nm, respectively. In the following, subscripts 1 and 2 refer to the two parts of light, and a and b are used to represent species a and b . Thus light intensity is divided into I_1 and I_2 corresponding to the wavelength 400 – 550 nm and 550 – 700 nm respectively. k_{ij} ($i=1, 2; j=a, b$) represents the light absorption spectrum of species j on part i of the light. The degree of complementarity between the two absorption spectra is controlled as follows:

$$k_{1a} = k_{2b} = 0.5 \times (1 + q) k_{max} \quad (5)$$

$$k_{2a} = k_{1b} = 0.5 \times (1 - q) k_{max} \quad (6)$$

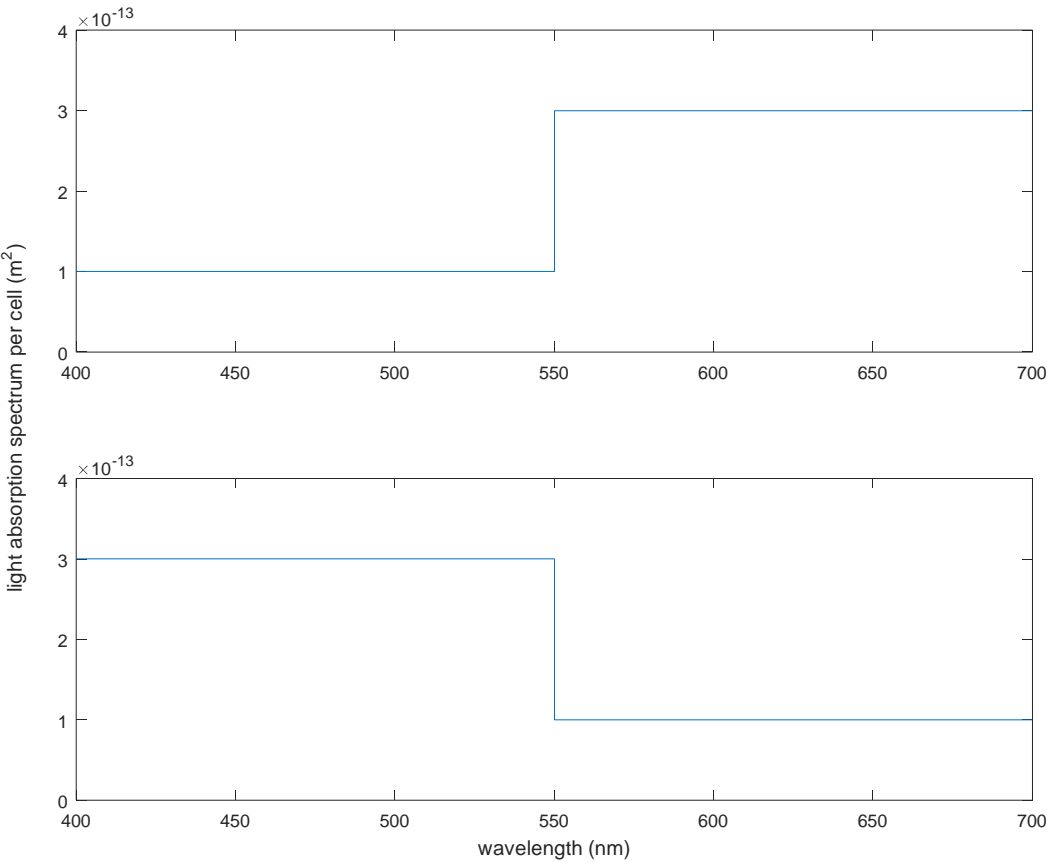
In equations (5) and (6), k_{max} represents the upper limit of light absorption spectrum. q is an adjustable parameter and imposes an increasing degree of complementarity when its value increases from 0 to 1. It should be noted that the purpose of these two equations is to create artificial absorption

spectra of two species with an easy-to-adjust degree of complementarity between the two spectra. At a given value of q , an absorption spectrum is defined for each species, which is applied to this species regardless of whether it is cultured alone or co-cultured with the other species. On the other hand, q is also used to achieve the adjustment of the degree of light absorption complementarity between the two species. An illustrative example corresponding to a medium level ($q = 0.5$) of complementarity is shown in Figure 2.

The default parameters that are used in the following work are shown in Table 1. The values for maximum specific growth rates, $p_{a_{max}}$ and $p_{b_{max}}$, are based on [17]. The upper limit of light absorption spectrum k_{max} is set so as to be consistent, in the order of magnitude, with the real light spectra reported in [17]. Given an average overall light absorption spectrum of $2 \times 10^{-13} \text{ m}^2 \text{ cell}^{-1}$ as shown in Figure 2, the optimal light absorption $\gamma_{i_{opt}}$ is set to correspond to an optimal light intensity (i.e. the level corresponding to the maximum growth rate) of $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (of photons). The literature reporting light saturation and inhibition in algal culture shows wide-ranging, species-dependent optimal light intensities [23]. In this work, the absolute level of the optimal light intensity (and the corresponding optimal light absorption) does not matter *per se*; what is of importance is the adoption of incident light intensities in the numerical simulations with reference to the optimal level of light intensity such that all the three regimes (linear, saturation and inhibition) are tested. Besides, values smaller than those in [17] were adopted for ϕ_a and ϕ_b which, in combination with the highest incident light intensity adopted, allow the growth rate model to predict a rather clear inhibition effect, to ensure that the impact of this effect is well captured in the simulation analysis.

144 Table 1. Default parameters used in numerical studies with the simplified absorption spectra.

Symbol	value	Units
ϕ_a	4×10^{10} (cells)	$\text{day}^{-1} (\mu\text{mol s}^{-1})^{-1}$
ϕ_b	4×10^{10} (cells)	$\text{day}^{-1} (\mu\text{mol s}^{-1})^{-1}$
z_m	7	cm
$\gamma_{i_{opt}}$	1×10^{-10} (per cell)	$\mu\text{mol s}^{-1}$
$p_{a_{max}}$	1	day^{-1}
$p_{b_{max}}$	1	day^{-1}
k_{max}	4×10^{-13} (per cell)	m^2



145

146 Figure 2. Illustration of simplified light absorption spectra of species *a* (upper plot) and *b* (lower plot).

147 From equations (1-3), the factors that may affect the yield of co-culture, hence the degree of

148 overyielding, include operation settings such as incident light intensity and cell density (which is largely

149 controlled via loss rate), as well as species traits of the microalgae involved, such as light absorption

150 spectrum, light use efficiency, and maximum growth rate. In this work, three different incident light

151 intensities, namely 40, 400 and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were considered. In reality, algal cultivation systems

may be operated with a diverse and wide range of light intensities ([24, 25]). As explained earlier, the absolute light intensities adopted in this work are not of importance; these three levels are selected only for the purpose of locating the system in the linear, saturation and inhibition regions of the $P - \gamma$ curve, respectively. Finally, light absorption spectra with various values of q were adopted in numerical experiments, together with the variation of the other parameters listed in Table 1.

2.3 Investigating a community of real species

Following the analysis using idealized absorption spectra, a community of two real species was considered to verify the findings from the former step. These species are chosen from the *synechococcus* group isolated from Baltic Sea named BS4 and BS5 as described in [17]. BS4 is a green picocyanobacteria containing pigment phycocyanin which absorbs red light whereas BS5 is a red picocyanobacteria with pigment phycoerythrin which can absorb blue and green light. Same as in [17], the reactor depth was set to $z_m = 7$ cm and an incident light intensity of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ (integrated over the range of 400 – 700 nm) was adopted. Monoculture of each species and co-culture of both species were simulated under two different loss rates to find out if and under which conditions overyielding can happen.

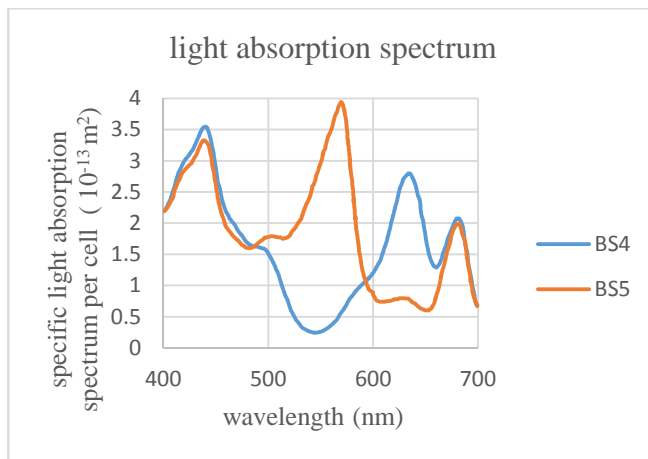


Figure 3. Light absorption spectra of two species [17]. BS4 and BS5 represent (green) picocyanobacteria and (red) picocyanobacteria, respectively.

3. Results and discussion

In this section, the results of numerical simulation of the system with the simplified spectra will be presented and analysed first, to show the impact of operation settings and species traits on overyielding potential when the system is subject to varying degrees of light absorption complementarity. The simulation results of the system with two real species will then be presented to offer a comparison.

3.1 Impact of operation settings

Assuming that the two algal species have the identical maximum specific growth rate and light use efficiency, simulation of the system was carried out to investigate the impact of different operation settings, in terms of incident light intensity and cell density. The overyielding potential affected by cell density (controlled via the loss rate) at different incident light intensities is indicated in Figure 4. The normalised overyielding level is calculated by the difference between the yield of co-culture and that of monoculture divided by the yield of mono-culture. Note that the latter is identical for the two species due to the fact that the only difference between the species a and b is their light absorption spectra which are symmetric to each other, therefore the two species will have an identical monoculture yield. A negative overyielding level implies the yield of the co-culture is worse than the monocultures, which is termed “underyielding”. From Figure 4, one can see that when incident light intensity is low (corresponding to the linear region of the light factor model, cf. equation 4), higher loss rates (lower cell density) and higher light absorption complementarity (i.e. q) tend to result in overyielding. When incident light intensity is increased to the light saturation and light inhibition region, underyielding can occur particularly when the loss rate (cell density) is sufficiently low (high). In such cases, there exists a lowest yield (with underyielding) at a certain level of q . It should be noted that in Figure 4, the range of the loss rate applied to the case with the lowest light intensity (the upper left plot) is lower than those for the other cases. This is because the low light intensity leads to slow growth, hence the loss rate needs to be low to avoid wash-out.

The above result shows that, in a system operating at a light-saturation or light-inhibition regime with a high cell density, the yield of a co-culture may become increasingly worse than the monoculture while the complementarity in light absorption between the two species increases; this trend gets reversed and eventually overyielding starts to occur and improve but only after the complementary exceeds a certain

level. To explain this rather counter-intuitive observation, Figure 5 and supplementary Figure 1 show plots of the photosynthetic productivity or the specific growth rate (defined as the product of the maximum specific growth rate and the light factor) of co-culture and mono-culture as a function of cell density, at different light absorption complementarity (i.e., q) and for a system operating in the light saturation regime. For each curve, its intersection with one of the two (low and high) loss rate lines represents the steady-state solution of the corresponding system. From Figure 5, one can see that at the steady state resulting from a higher loss rate, which gives rise to a lower cell density, the yield of the co-culture system is higher than that of the mono-culture system; this gap tends to increase with q . In terms of the specific growth rate, the monoculture would have a lower value than that of the co-culture at the steady-state cell density of the latter, indicating that the former would be less productive. Moving to the lower loss rate which corresponds to a higher cell density, which is the case leading to the counter-intuitive observation as presented earlier, one can see that the steady-state cell density of the co-culture is lower than that of the mono-culture at low q values, with this gap initially increasing with q and then reversed afterwards. At a point where the co-culture yields less than the monoculture, the specific growth rate of the mono-culture would be higher than that of the co-culture at the steady-state cell density of the latter, indicating that the former would be more productive in this case.