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Light distribution and spectral composition within cultures of micro-algae: Quantitative modelling of the light field in photobioreactors

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ABSTRACT

Light, being the fundamental energy source to sustain life on Earth, is the external factor with the strongest impact on photosynthetic microorganisms. Moreover, when considering biotechnological applications such as the production of energy carriers and commodities in photobioreactors, light supply within the reactor volume is one of the main limiting factors for an efficient system. Thus, the prediction of light availability and its spectral distribution is of fundamental importance for the productivity of photo-biological processes.

The light field model here presented is able to predict the intensity and spectral distribution of light throughout the reactor volume based on the incident light and the spectral characteristics of the photosynthetic microorganism. It takes into account the scattering and absorption behaviour of the micro-algae, as well the adaptation of the biological system to different light intensities.

Although in the form exposed here the model is optimized for photosynthetic microorganism cultures inside flat-type photobioreactors, the theoretical framework is easily extensible to other geometries. Our calculation scheme has been applied to model the light field inside *Synechocystis* sp. PCC 6803 wild-type and Olive antenna mutant cultures at different cell-density concentrations exposed to white, blue, green and red LED lamps, delivering results with reasonable accuracy, despite the data uncertainties. To achieve this, *Synechocystis* experimental attenuation profiles for different light sources were estimated by means of the Beer-Lambert law, whereby the corresponding downward irradiance attenuation coefficients $K_d(\lambda)$ were obtained through inherent optical properties of each organism at any wavelength within the photosynthetically active radiation band. The input data for the algorithm are chlorophyll-specific absorption and scattering spectra at different mean acclimatisation irradiance values for a given organism, the depth of the photobioreactor, the cell-density and also the intensity and emission spectrum of the light source.

In summary, the model is a general tool to predict light availability inside photosynthetic microorganism cultures and to optimize light supply, in respect to both intensity and spectral distribution, in technological applications. This knowledge is crucial for industrial-scale optimisation of light distribution within photobioreactors and is also a fundamental parameter for unravelling the nature of many photosynthetic processes.

1. Introduction

1.1. Light research in aquatic ecosystems

1.1.1. Introduction to optics in biology

Photosynthesis is a very active research field in the life sciences due to the crucial importance of photosynthetic organisms as the fundamental source of all biomass in our planet. Particularly, much research has been done in understanding how light behaves inside different water bodies, such as inland, coastal and oceanic ecosystems.

Concurrently, bio-optical researchers have developed several methodologies to estimate optical properties. In the year 1961 Preisendorfer defined the inherent (IOPs) and apparent optical properties (AOPs) of water bodies, founding optical oceanography [1]. Relating IOPs and AOPs have been an ongoing effort since then, and different authors have studied, experimentally as well as theoretically [2], the optical characteristics of water and cell suspensions as a function of water body features and metabolic variables such as the energy stored by algae upon light conditions [3].

But oceanic optics is not the only field of interest in the study of light interaction with microorganisms. During the last 30 years, more interest has progressively been devoted to the development of closed photobioreactors (PBRs), aimed at the production of many substances

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of interest ranging from nutra- and pharmaceuticals, to bioenergetic compounds [4], [5]. As dense cultures are preferred to maximise production, light is normally the limiting factor to obtain a cost effective PBR operation. Although dense suspensions are a priori more appropriate for an efficient PBR utilisation [6], too concentrated cultures may increase operating costs [7] and completely deplete the system of light in most the external layers [8] as well. Therefore, optimisation of illumination conditions and cell density is required for improving overall photosynthesis performance and to minimise dark respiration and thus for achieving an optimal design of large-scale photobioreactors [9].

From the point of view of light propagation, there are important differences between the conditions in open waters or inside a PBR aqueous phase. The use of artificial light sources in many PBR setups, unnatural light cycles, the geometry of the arrangement itself and its inherent limitation in culture depth, not present in most open waters, are just some of the differentiating factors. A crucial topic is the question of stratification. Whilst in open waters a given equilibrium stratification is established within the photic zone and substantial differences may be found in microorganism concentration and composition depending on depth, inside a PBR efforts are usually oriented towards obtaining a good mixing so that the photosynthetic cells can rapidly move towards the external and internal zones of the reactor. Accordingly, the culture inside the PBR volume is usually regarded as being homogeneous.

Regarding the strategies to describe light distribution within water bodies, authors have either used algorithms that calculate the light field based on the radiative transfer equation describing light-matter interaction [10] or have applied stochastic methods such as Monte Carlo simulations [11,12], which allow researchers to statistically follow the fate of individual photons within the medium. Relevant works based on this strategy have been published in the last decades. In this regard, in some cases the light field prediction is linked with experimental cell growth [13,14] or coupled biomass production is modelled following a classical growth law such as Monod-type [15]. Several applications on different reactor shapes such as torus photobioreactors [16] or open ponds [17] can be found.

In our approach we aim at creating a procedure in between the simple light models and exceedingly detailed simulations in order to get a holistic view of the interaction of light and biomass based on the IOPs of the cells of interest, which has not been described in literature and is novel to the field. To do so, we will derive a relationship connecting the light field profile within a PBR suspension knowing the cell density, lamp emission spectrum, culture depth, absorption and scattering coefficients of the culture acclimatised to different light intensities. Making some simplifying assumptions we arrive at an expression that can be easily solved and can even give rise to an analytic relationship between operating parameters of the culture and includes in an implicit manner photo-adaptation of the cells. Furthermore, we have tested our scheme using information from two sources, completed with our own experiments, on two different strains of *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis*), the wild-type and the Olive mutant. The latter is a strain with truncated phycobilisome structure, where the phycobilisome core is present but the rods are absent [18].

The model is able to predict the light attenuation caused by cultures in a considerable range of optical densities and light sources. Besides, the methodology proposed in this work follows a semi-mechanistic calculation procedure that can be generalised to other microorganisms and reactor geometries, whereas other published contributions are merely empiric fits or assume that absorption is the only factor for light attenuation. Moreover, this methodology is also capable of predicting spectral composition of light within the photic zone.

In the following sections we will explain the main features of our modelling approach and its assumptions: Section 2 exposes the experimental information and underlines how our method can be used in practice combining existing information with novel experiments. Section 3 discusses the results and highlights some interpretations that can be obtained from these analyses. Section 4 contains the conclusions and further outlook of our work.

1.1.2. Light spectrum influence in photosynthetic mechanisms

As stated before, light spectral composition in a PBR is sometimes not just a given condition, but can be selected and optimised. For an optimal selection of the light source, it is not only important to consider lamps whose emission peaks overlap the cell absorption spectra, but also other factors such as scattering, quantum yield and excitation balance between both types of photosystems [19].

Moreover, not only the light absorption capacity of the cells but also its efficiency in converting the captured photons into usable energy has to be taken into consideration. In this regard, the action spectrum represents the quantum yield of this efficiency upon light wavelength. It is important to note that the action spectra can vary depending on the pre-illumination conditions [20] or if supplementary light is applied. In the latter case, if cells are not exposed to some background light, the action spectrum can differ greatly from the absorptance spectrum in some wavelengths [21]. In other words, when using a monochromatic light source, the spectrum of the chosen lamp has to provide a balanced amount of quanta for both types of photosystems.

While it is common practice to study how white light affects growth in photosynthetic microorganism cultures, including mechanistic approaches for the photo-adaptation phenomenon [22], less research has been performed on how other types of light sources impact photosynthesis rates and related mechanisms. Specifically in cyanobacteria, some contributions can be found regarding light colour effect on oxygen evolution [23], redox state of the plastoquinone pool [24], growth [25] in Synechocystis, biomass composition of *Arthrospira platensis* [26] or areal biomass productivity in *Chlamydomonas reinhardtii* [27]. In Zavrel et al. research [25] and Markou contribution [26], blue light led to lower growth than red in both species, whereas in Ref. [27] yellow light promoted the highest productivity. Available irradiance as a function of the remaining wavelengths can shed light on real photosynthesis rates as quanta are absorbed by pigments which have specific absorption spectra on one side while part of the light is scattered in a spectrally dependent way. Particularly in *Synechocystis* cultures, blue is the most scattered colour and red the least [28], though this phenomenon relies on the type of organism and the aquatic environment [29].

Delving deeper in spectral composition of light publications, it must be noted that there are few experimental works which describe the wavelength dependent light distribution along the optical path-length. Measured spectra of remaining light within PAR range at different depths in cyanobacterial cultures of *Spirulina platensis* [8], suspensions of *Chlamydomonas reinhardtii* [16] and in *Microcoleus chthonoplastes* mats [30] are among the few. However, knowing the light field inside PBR cultures would help in designing large-scale flat-type PBRs and predicting growth conditions for maximal photosynthesis rates, e.g. optimal cell density and depth for given illumination conditions and species.

In summary, it is common to model and present photosynthesis as a function of the total white light intensity applied in the system as this approach is sufficient for validating general culture properties. However, knowing the spectral composition of light is necessary to deeply understand its effect on many photosynthetic processes.

1.2. Modelling framework definition

1.2.1. Inherent optical properties: definition and measurement

The two basic IOPs [31], the absorption and scattering coefficients (Table 1), are defined on the basis of an imaginary, infinitesimally thin plane, parallel layer of medium, illuminated at right angles by a parallel beam of monochromatic light. AOPs, such as the different coefficients describing vertical attenuation, are properties of the radiation field depending not only on intrinsic features of the water body but also on the angular distribution of the light within the system as well as the depth.