

Light requirements in microalgal photobioreactors: an overview of biophotonic aspects

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Abstract In order to enhance microalgal growth in photobioreactors (PBRs), light requirement is one of the most important parameters to be addressed; light should indeed be provided at the appropriate intensity, duration, and wavelength. Excessive intensity may lead to photo-oxidation and -inhibition, whereas low light levels will become growth-limiting. The constraint of light saturation may be overcome via either of two approaches:

increasing photosynthetic efficiency by genetic engineering, aimed at changing the chlorophyll antenna size; or increasing flux tolerance, via tailoring the photonic spectrum, coupled with its intensity and temporal characteristics. These approaches will allow an increased control over the illumination features, leading to maximization of microalgal biomass and metabolite productivity. This minireview briefly introduces the nature of light, and describes its harvesting and transformation by microalgae, as well as its metabolic effects under excessively low or high supply. Optimization of the photosynthetic efficiency is discussed under the two approaches referred to above; the selection of light sources, coupled with recent improvements in light handling by PBRs, are chronologically reviewed and critically compared.

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Introduction

Microalgae are microorganisms characterized by a high productivity per unit area when compared with such other photosynthetic organisms as higher plants. This outstanding photosynthetic efficiency results from a reduced number of internally competitive physiological functions, fast reproduction cycles, limited nutrient requirements, and adaptation to a broad range of temporal and spectral irradiances (Gordon and Polle 2007). Furthermore, a few microalgal cultures (e.g., *Dunaliella*, *Spirulina*, and *Chlorella* spp.) are relatively prone to scale-up in photobioreactors (PBRs), where it is in principle possible to provide optimal nutrient levels on

a continuous basis, adjust harvest rates to keep the culture concentration at preset levels, and control cell composition without decreasing overall productivity.

A broad list of applications of microalgal metabolites have been described and discussed in the technical literature. Those with commercial expression encompass food additives, pigments, healthy foods, fine chemicals and wastewater treatments. Production of several active compounds—viz. hydrocarbons, isotopes and polysaccharides, as well as compounds possessing antifungal, antitumor, antibacterial and antiviral activities, is currently under active scrutiny; furthermore, utilization of microalgae for CO₂ fixation, removal of nitric oxide from flue gas, production of lipids for biodiesel, and recovery of heavy metals from effluents already entail a potential for commercial applications (Borowitzka 1997; Plaza et al. 2009; Kumar et al. 2010).

Despite their promising biotechnological uses, microalga-based applications that have reached industrial scale are relatively scarce. The main reason underlying such a low practical impact derives from the high production costs involved, given the current market prices of the products of interest; therefore, there has been an impetus toward decreasing the costs of operating PBRs with microalgae.

The question of which are the major bottlenecks in microalgal growth and metabolite production is of crucial importance for the development of economically feasible, large-scale cultivation systems. Among the abiotic (e.g., light, temperature, gases, pH, and nutrients), biotic (e.g., pathogen contamination and competition with other microorganisms) and operational factors which play a role in PBR engineering, light outstands as a key processing parameter—especially owing to the difficulties associated with its control (viz. assurance of stability throughout time and uniformity throughout space); most

of the so-called novel bioreactors, claimed to have been specifically developed for microalgae, do in fact attempt to overcome the constraints related with control of said parameter.

In contrast to the increasing research efforts currently underway encompassing genetic improvement of native freshwater and marine strains for specific applications, the modes of supply of an adequate amount and quality of light to microalgal cells are still rather conventional. The light issue has sometimes been circumvented *via* growing the microalgae heterotrophically, yet not all microalgae (or microalgal products, for that matter) can or should be produced in this way; therefore, PBR engineering should be targeted at attaining technological leaps in the near future, pertaining to much more efficient cultivation modes. This review covers this topic, by discussing reported approaches and suggesting future streamlines.

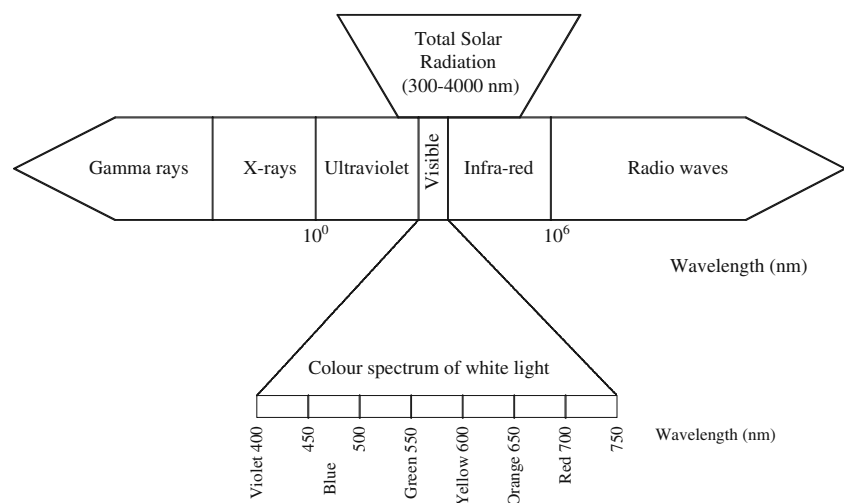
Light supply and utilization

In order to effectively design a PBR able to provide the correct light—in both quantity and quality, and efficiently transporting it to the vicinity of microalgae to allow photosynthesis to occur, it is essential to understand the fundamentals of the photosynthetic process. The nuclear topics in this particular are briefly presented below.

Nature of light

Light (or visible light) is but one segment of the electromagnetic radiation spectrum, as depicted in Fig. 1. Radiation can be physically treated as particles—quanta (or photons), or instead as waves; either of these theories can account for different pieces of evidence describing the

Fig. 1 Whole electromagnetic spectrum, with detailed spectral pattern of visible light



behavior of light. Each photon consists of a discrete portion of energy, E , given by

$$E = h\nu, \quad (1)$$

where h is Planck's constant (6.626×10^{-34} J s) and ν is the wave frequency. Equation 1 can be rewritten as

$$E = hc/\lambda, \quad (2)$$

where c is the speed of light in vacuum (3×10^8 m s⁻¹) and λ is the wavelength of light (m). Inspection of Eq. 2 indicates that the energy specific content is inversely proportional to the wavelength features: shorter wavelengths have higher energy, whereas longer wavelengths have lower energy per photon.

The various types of radiation differ in their wavelengths, and consequently in the amounts of energy carried by their individual quanta. Radiation of 750 nm and above has an energy content that is too low to mediate chemical changes; hence, radiant energy absorbed in this range will only appear as thermal effects. Conversely, radiation of 380 nm and below brings about ionizing effects. Between 380 and 750 nm, the energy content is sufficient to produce chemical changes in the absorbing molecules, as happens throughout the photosynthetic pathways prevailing in microalgae (Kommareddy and Anderson 2003). Hence, visible light is the main source of energy for autotrophic microalgae to produce organic compounds using the photosynthetic process.

Light harvesting and transformation

Pigments

Most materials absorb preferentially certain light wavelengths. Any compound that absorbs specific wavelengths exhibits consequently a distinctive color, and is termed pigment (Mauseth 1991). Energy absorption by photosyn-

thetic organisms is thus dependent on the chemical nature of their constitutive pigments. The major pigment groups present in microalgae—viz. chlorophylls, phycobilins, and carotenoids (carotenes and xanthophylls), are described in Table 1 (Masojidek et al. 2004).

Chlorophylls account for the most important group; their structure comprises a phytol tail and a porphyrin ring. The former appears dissolved in the membrane lipids of the chloroplast, whereas the alternating single and double bonds of the porphyrin moiety act as antenna to capture light—with the magnesium atom at the center carrying the electrons necessary for the photosynthesis reactions to take place.

Within the aforementioned major group, the most important molecule is chlorophyll *a*. Accessory pigments include chlorophylls *b* and *c*, as well as carotenoids; the former absorb other wavelengths of the radiation spectrum and pass their energy onto chlorophyll *a*, whereas the role of carotenoids seems to be absorption of excess light (and thus protection of chlorophyll integrity). Carotenoids are usually red, yellow or orange, so they do not absorb light in those regions, but instead in the violet/blue and blue/green regions of visible light (Kommareddy and Anderson 2003). Phycobilins are water-soluble pigments, consisting of many closely related compounds—e.g., phycocyanin, a blue pigment present in blue-green microalgae.

Photosynthesis

As the name implies, photosynthesis is a process that uses light energy to chemically synthesize molecules. The basic chemical equation can be summarized as:



Its positive heat of reaction (2,814 kJ) indicates that energy is required for the reaction to proceed, which is

Table 1 Photonic features of major pigments in microalgae

Pigment group	Color	Ranges of absorption bands (nm)		Pigments
Chlorophylls	Green	450–475 630–675	Hydrophobic	Chlorophyll <i>a</i>
				Chlorophyll <i>b</i>
				Chlorophyll <i>c</i> ₁ , <i>c</i> ₂ , <i>d</i>
Phycobilins	Blue, red	500–650	Hydrophilic	Phycocyanin
				Phycocerythrin
				Allophycocyanin
Carotenoids	Yellow, orange	400–550	Hydrophobic	β -Carotene
				α -Carotene
				Lutein
				Violaxanthin
				Fucoxanthin

provided by incident radiation; although the first steps of light capture by plants are close to maximum efficiency, later steps are less efficient, so the actual conversion of solar energy to biomass will eventually be as low as 1–8%. Microalgae seem to absorb light much more efficiently than land plants; photosynthetic efficiencies of ca. 20% have been attained in cultures of *Chlorella* sp., *Phaeodactylum tricorutum*, and *Tetraselmis suecica* (Packer 2009).

In the first stage of the photosynthetic process, light has to be intercepted by absorbing substances; since chlorophyll *a* and other pigments differ in their absorption spectra, the combination prevailing in each type of microalgal cells allows absorption in a far broader spectral range than if photosynthetic systems consisted of only one such pigment. These pigments are intrinsically associated with specific proteins, thus forming highly complex Light Harvesting Protein-Chlorophyll *a* systems (LHPC), or antenna—which are embedded in the thylakoid membranes; all together, as well as with other light-absorbing cellular structures and compounds, they form a cloud aimed at impinging light in a cell (Dubinsky et al. 1995).

The photosynthetic process occurs along two main steps: light-dependent reactions, which originate energy and reducing power, via ATP and NADPH as intermediates, respectively; and dark reactions, which constitute the Calvin cycle, in which those intermediates react with CO₂ to produce glucose. During this set of reactions, the carbon atom of CO₂ is reduced—a process that requires a source of electrons and a source of energy: the former is water, whereas the latter is light.

All chlorophyll molecules and electron carriers working together in the energy capture process must stay very closely packed—so that when light excites electrons in chlorophyll *a*, they will instantly react with the proper molecule before losing their energy by fluorescence. The granule where those molecules are packed together is termed a photosynthetic unit; typical thylakoid membranes are filled with millions of these granular arrays. Light reactions occur in two types of photosynthetic units—photosystem I (with almost pure chlorophyll *a*) and photosystem II (in which chlorophyll *b* is also present to a significant level); they use energy associated with slightly different wavelengths (viz. 680 and 700 nm). These two photosystems work together in transferring electrons from water to NADPH.

The photosynthesis process accordingly starts when a photon impacts a chlorophyll *a* molecule: it is either reflected, transmitted or absorbed by said molecule. If absorbed, the molecule is excited from a stable ground state (S₀) to an excited state (S₁) that depends on the energy of the incident wavelength. If the incident wavelength is 450 nm or lower, the chlorophyll molecule will be excited to the second singlet (or excited) state (S₂), and conse-

quently will need to lose some energy afterwards, as fluorescence or heat, in order to fall back to S₁. The energy lost as heat or fluorescence is not indeed used in photosynthesis, so it contributes unfavorably to light use efficiency.

The jump from S₀ to S₁ requires an energy input close to that conveyed by photons characterized by 680–700 nm, depending on the photosystem at stake (I or II). Once the electron is on S₁, it may either: (1) be transferred to a photochemical process; (2) move to the triplet state (a process that entails rearrangement of the excited state, thus leading to spin reversal—and possibly to photooxidation); (3) or lose energy, as heat or fluorescence, and thus move back to S₀. Therefore, excitation of chlorophyll molecules should occur in the vicinity of the first singlet state (680–700 nm), so that the energy transfer from the incident photon to the electron in the molecule will be more effective, and accompanied by minimal energy dissipation.

The ultimate rate-limiting processes in photosynthesis are the dark reactions. There is no consensus on what can be considered as the appropriate duration for a dark/light cycle; since naturally growing microalgae are necessarily exposed to dark at night, it is assumed that dark periods are necessary. On the other hand, long dark periods (i.e., of the order of magnitude of several hours) generally result in biomass loss, as well as decline in growth rates, because microalgae switch to respiration processes; several authors have indeed suggested that a suitable dark period should be of the order of milliseconds (Kommareddy and Anderson 2004)—which would be more appropriately seen as an intermittent cycle.

Photosynthetic efficiency

Time scales characterizing photosynthetic processes can be divided in three ranges: primary photochemistry, electron shuttling and carbon metabolism. The former occurs from light harvesting through charge separation in the reaction centers, within pico- to nanosecond-periods. Reactions involving shuttling of electrons between photosystems I and II (dark reactions) are slightly slower—and take micro- to millisecond-periods. Finally, carbon metabolism within the chloroplast occurs in second-scales, whereas sucrose metabolism and enzyme activation may take up minutes (Tennessen et al. 1995). These three processes can be deliberately uncoupled, by providing pulses of light characterized by the appropriate length within the time range of each process.

Once a photon is absorbed, the system needs 1–15 ms to reset itself, prior to being ready to receive another photon. Absorption of a photon is almost instantaneous, so the time necessary for a microalgal cell to remain in the lit region of a PBR is a function of how long it takes

for a photon to impact the absorbing pigment in its antenna. Such a situation is a function of the incident light flux, the depth within the PBR and the amounts of absorbing pigments available in the antenna region (Kommareddy and Anderson 2004). In view of the relative magnitude of the aforementioned time scales, the turnover time of the photo-synthetic unit (PSU), or photosynthetic reaction center, is given by the dark reaction time for practical purposes.

The light-dark cycle period, which is determined by the travel time of cells between the dark and lit portions along the PBR, should accordingly be made as short as possible; this usually means an optical path of 0.5 to 1.0 cm (Richmond et al. 2003).

As light is the basic energy source for microalgae, their light harvesting efficiency is of crucial importance in engineering efforts encompassing those microorganisms. One important indicator thereof is the photosynthetic efficiency (PE)—defined as the fraction of available incident light within the solar spectrum that is stored as chemical energy in biomass; however, there is no general consensus on how to calculate its actual value. In practice, especially in long-term cultures, PE is normally below 6.5%; even under optimal conditions, no more than 40% can typically be attained, so attempts to increase PE are a promising approach in the quest to enhance microalgal productivity.

To estimate PE for a selected species in a given PBR, one departs from calculation of the illuminated surface area per unit volume of the culture, A_v ; for instance, in a vertical tubular reactor (one of the most popular bioreactor configurations for microalgae), one has

$$A_v = \frac{2r_o}{\pi r^2} \quad (4)$$

where r and r_o are the inner and outer radius of the tubes, respectively (Pirt et al. 1983).

Application of the microbial-growth energy equation after several modifications (Pirt 1982), coupled with the assumption of an essentially nil maintenance coefficient, one gets:

$$Y_G = \frac{\mu X}{\phi I_o A_v}, \quad (5)$$

where Y_G is the maximum growth yield on light, μ is the specific growth rate, X is the biomass concentration, ϕ is the fraction of photosynthetically available light, and I_o is the total incident light.

In a reactor operated under a constant A_v and a given I_o , it is possible to adjust μ and X so as to achieve the maximum Y_G —which then also leads to the maximum PE. If a proper reactor design is sought, it is thus crucial to obtain the maximum biomass yield at the expense of light

actually available. This is in particular the case of artificially illuminated bioreactors—where I_o depends on the nature, intensity and relative position of the light source, all of which are under one's control.

Effects of excessively low and high light supply

Although light is required for photosynthesis, too low or high levels thereof will entertain serious disadvantages—arising from the intrinsic nature of that metabolic process. The underlying mechanisms will be discussed below to some length.

Response of photosynthesis to light intensities

Light is necessary for microalgal photosynthesis, yet excessive or insufficient incident light constrains optimal performance—in terms of biomass or metabolite yields. Photosynthesis (and the corresponding production of biomass) depends indeed on the photonic flux—as graphically represented in Fig. 2 (Goldman 1980). The biomass term is often expressed as chlorophyll a content, total pigment content, organic carbon content, organic nitrogen content or dry weight—as they correlate relatively well with each other. This type of curve helps one to predict and model photosynthesis, while allowing an assessment of variations in photosynthetic physiology: for instance, one can obtain the maximum photosynthetic rate (P_{max}), and PE as the initial slope of the photosynthesis versus irradiance curve.

Three main areas can be distinguished in Fig. 2: (1) a light-limited region, in which the photonic input rate is fully applied in photosynthesis, so PE rises with increasing

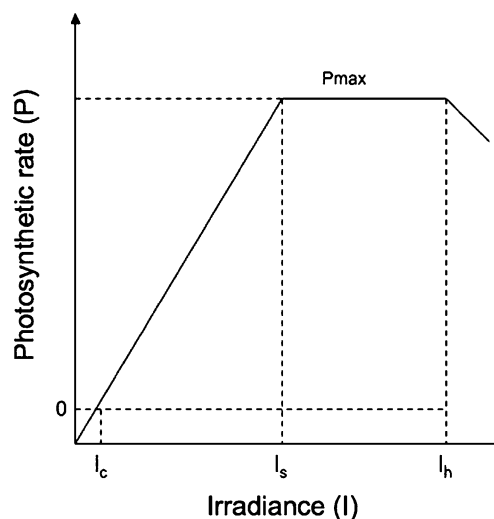


Fig. 2 Typical photosynthesis (P) vs irradiance (I) curve for microalgal cells (the various symbols are explained in the main text)

irradiance; such a region is delimited by the light intensity at which microalgal cells begin to grow (i.e., the compensation light intensity, I_c), and the light intensity at which no further increase in growth occurs upon increasing light intensity (i.e., the light saturation, I_s); (2) a light-saturation area, in which the photosynthetic processing capacity of the culture attains its maximum value, and the excessive photonic flux provided to the culture is dissipated as heat or fluorescence; and (3) a photo-inhibition region, in which increases in light intensity become injurious—and are expressed initially by a decrease in growth rate, eventually attaining photo-damage and even leading to culture death, after a point denoted as photo-inhibition (I_h).

In stirred PBRs, the photosynthetic performance of microalgal cultures under bright sunlight usually switches between the three areas, although with a dominance of (1) and (2). Photosynthesis and photo-inhibition occur preferentially in cells near the culture surface, while mutual shading of cells causes steep gradients of light intensity within the culture—with rather low light values available to cells well apart from the culture surface (Neidhardt et al. 1998). Hence, microalgal cultures even growing under full sunlight typically undergo suboptimal light/biomass conversion efficiencies. The reason for this inefficiency is that, in fully pigmented cells under high photon flux densities, the rate of photon absorption by the antenna chlorophylls of the first layers of cells in the culture exceeds the rate at which photosynthesis can occur to fully utilize their energy—thus producing non-photochemical quenching of excess photons, or even inhibition of photosynthesis. This means that up to 50–80% of absorbed photons may be wasted, thus reducing solar conversion efficiency and cellular productivity to relatively low levels.

Photo-inhibition and photo-oxidation

At light flux values beyond the plateau region represented in Fig. 2, photosystem II can be rapidly damaged, so bioproductivity will decrease as the photonic flux is further raised—an effect known as photo-inhibition. Despite the empirical observation that microalgal tolerance to light flux occurs at 200–400 $\mu\text{mol}_{\text{photons}}\text{m}^{-2}\text{s}^{-1}$, such a threshold does not constitute a strict limit; indeed, there are reports attesting flux tolerances up to 5,000 $\mu\text{mol}_{\text{photon}}\text{m}^{-2}\text{s}^{-1}$ without significant flux saturation, when using light-emitting diode (LED) sources and instantaneous (but discontinuous) light fluxes (Gordon and Polle 2007).

The temperature of the medium also influences light intensity requirements for optimal growth (Kommareddy and Anderson 2004): microalgae do in fact tolerate higher irradiances at temperatures closer to their optimum growth temperature (Borowitzka 1998). Since outdoor microalgal

cultures are exposed to daily changes in environmental conditions, especially irradiance and temperature, limiting and possibly inhibiting values for photosynthesis can be recorded. On the other hand, de-synchronization between them may induce unwanted stress; Vonshak et al. (1982) demonstrated this pattern in *Spirulina* sp. grown in outdoor raceway ponds, in which early morning low temperatures induced photo-inhibition.

As mentioned before, microalgae undergo photooxidation when a chlorophyll molecule is excited to the triplet state; this is a very unstable form, which reacts with oxygen and transfers energy to it while falling to the ground state. The excited oxygen then reacts with fatty acids to form lipid peroxides—which are detrimental to the cell membrane, and can even lead to cell death.

Photo-acclimation

Because of the relatively rapid and wide changes in ambient light to which they are subjected, microalgae have a remarkable capacity for photo-acclimation; this process consists of a series of interrelated physical, biophysical, biochemical and physiological changes that aid microalgal cells optimize their use of available light (Dubinsky et al. 1995). Damage by excess light depends to a certain degree on the state of photo-acclimation: cells acclimated to relatively low light (i.e., shade-adapted) prior to exposure to high intensity radiation will become photo-damaged at a lower irradiation dose than cells which have been high light-acclimated. Therefore, in batch cultures grown outdoors, it is advisable to previously acclimate the inoculum to high light values. On the other hand, when the cellular inoculum is small, initial irradiance should also be low, otherwise photo-damage may cause culture collapse.

Optimization of photosynthetic efficiency

Two major routes can be devised to increase the level and effectiveness of light utilization by microalgae: action on the receptor via genetic engineering, or action on the source via light engineering. Either one possesses advantages and shortcomings, as detailed next.

Genetic engineering

Measurement of quantum yields of microalgal photosynthesis under limiting light intensity has revealed a photon conversion efficiency of 82%, thus suggesting that their photosynthetic apparatus can use almost all photons absorbed; however, when similar cultures of microalgae were grown under full sunlight, they exhibited much lower

efficiencies (Mitra and Melis 2008). Such a discrepancy may be justified by the large array of light-absorbing chlorophyll antenna molecules present in photosystems I and II of microalgae, which allow the photon capture system to be far more efficient than the remaining photosynthetic process; hence, up to 80% of the photons absorbed at high solar irradiances may be dissipated afterwards as heat or fluorescence, thus decreasing the observed photosynthetic productivity.

In attempts to surpass the aforementioned limitation, it is necessary to minimize light absorption by individual cells, and concomitantly permit increased transmittance of radiation through the culture, so that cells deeper inside the culture will receive enough light. This can be achieved by reducing the size of chlorophyll antenna molecules in the chloroplast photosystems. In fact, studies using photosystem mutants of *Chlorella pyrenoidosa* showed that a small photosynthetic unit size (Chl *a*/P700) had a higher photosynthetic activity, on a cell basis (Lee 1990). Later, Nakajima et al. (2001) reported 1.5-fold improvement in photosynthetic productivity when comparing wild type cultures with a mutant (obtained by UV mutagenesis, and possessing a lower level of light-harvesting pigment) of *Chlamydomonas perigranulata*. Genetic engineering tools, coupled with sensitive absorbance-difference kinetic spectrophotometry, may in principle permit one to partially truncate the chlorophyll antenna size of a microalga in a controlled fashion, thus alleviating over-absorption of incident light by individual cells in high-density cultures—and concomitantly minimizing dissipation of irradiance. This feature can also diminish the cell-shading that occurs with normally pigmented wild type cells, thus allowing a more uniform illumination of the whole cells, especially in cultures characterized by high biomass densities. Additionally, these improvements can be extended to the efficiency of carbon fixation reactions (Gordon and Polle 2007; Beckmann et al. 2009).

Light engineering

Recall that the intensity of a light source is directly associated with the number of photons that are emitted per unit area. The energy associated with photons with a wavelength of 680 nm is the exact energy level required by chlorophyll *a* to initiate photosynthesis; as this wavelength is near the longest wavelength of visible light, most of the spectrum of visible light has sufficient energy per photon to support photosynthesis (Kommareddy and Anderson 2003). Due to the high light-harvesting efficiency of chlorophyll, microalgae absorb essentially all light that reaches it, even though not all photons will be used; the wider the spectrum, the lower the light utilization efficiency, with consequent decreases in maximum growth yield.

An alternative to light supplied on a continuous basis and over extended periods of time is to use intermittent light; theoretical studies on its effects upon photosynthesis showed that high intensity light will be used with increased efficiency if made available as short flashes, separated by long dark periods. Contreras et al. (1979) found that the blue-green alga *Oscillatoria* sp. exhibits a maximum growth rate under a radiation intensity of 50 W m^{-2} , yet said maximum shifted to 75 W m^{-2} when a dark period of 0.1 s was introduced. According to those authors, during the dark phase the thylakoid discharges the excessive voltage accumulated because of high irradiation; such a discharge is accomplished by chlorophyll-carotene transfer of energy (a phenomenon that is temperature-dependent).

Flashing is often viewed as an on-off character imposed onto an otherwise continuous light source—via eliminating part of it at regular intervals; however, pulsating also involves condensation of the whole energy into shorter periods, nominally at no overall energy compromise. The averaged intensity thus comprises periodically alternating high-intensity and dark-cycle periods; and the “on” fraction of the on-off cycle—in terms of duration and intensity, is thus a processing parameter prone to optimization (Gordon and Polle 2007).

The first experiments encompassing use of flashing light date back to 1932, with the pioneering work of Emerson and Arnold; these researchers found that the maximum rates of O_2 production and CO_2 uptake, obtained under a sequence of very short flashes, were the same as those under continuous light with the same overall intensity. Therefore, they concluded that cells undergoing photosynthesis do not require continuous illumination. Later, Tennessen et al. (1995) compared the response of photosynthesis under continuous light with that when the same total photon flux was delivered as intense pulses, lasting just 1% of the overall time; they used pulses of up to $5,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and frequencies ranging from 0.5 to 5,000 Hz. Their results revealed that the photosynthetic apparatus was able to integrate the pulsed light and use it as efficiently as if the light were delivered in a continuous mode. When pulses were extended, photosynthesis under pulsed light fell below that under continuous light, probably because each light pulse delivered more photons than those that could be turned over by, or stored in the electron transport system.

More recently, Park and Lee (2001) reported that cultures of *Chlorella kessleri* at 1×10^7 cells mL^{-1} (i.e., high-density cultures) experienced enhancements in specific oxygen production rates under flashing light, when compared with continuous light. However, such enhancements disappeared at lower cell densities, viz. 1×10^6 cells L^{-1} , thus suggesting that instantaneous high light intensity caused photo-inhibition of microalgae at lower cell densities. At higher

cell densities, an increased photosynthetic photon flux may help photons penetrate deeper into the culture, thus reducing mutual shading—which would accordingly increase the volume of the photon-rich zone, and hence the ratio of cells that would receive enough light to perform photosynthesis. In general, proper flashing light under high cell concentrations may increase efficiency of light utilization, and thus enhance growth rate and metabolite productivity (Kim et al. 2006; Grobbelaar 2009).

The effects of flashing light upon production of secondary metabolites was studied in detail by Kim et al. (2006) using *Haematococcus pluvialis* as model microalga. Their experiments revealed that, when light energy was supplied as short and intense flashes, synthesis of the red ketocarotenoid pigment astaxanthin was induced more efficiently than when the same amount of light energy was provided continuously.

From the evidence made available to date, it can be concluded that the rate of photosynthesis under flashing light is never above that under continuous light of the same intensity; however, the efficiency in utilization of light by microalgae tends to be higher when intermittent light is used. Furthermore, it is not necessary to ensure a precise flash time to achieve a considerable increase of efficiency, provided that the incident light intensity is high. One concept related with this issue is the Critical Cell Density, CCD (usually expressed as cell mL⁻¹), which is the maximum cell concentration that can be attained in microalgal cultures without measurable mutual shading; it is a function of the average cell volume and light illumination area, and can be calculated as (Park and Lee 2001)

$$\text{CCD} = \frac{A_i}{\frac{\pi D_c^2}{4} \times V} \quad (6)$$

where A_i is the illuminated area, D_c is the average diameter of an individual cell, and V is the volume of the culture. Obviously, when the number of cells present is such that the total area occupied thereby overcomes the overall exposed area of the culture, mutual shading will take place. In view of the above considerations on the ability of light penetration, flashing light will be useful only with cell densities above CCD.

Selection of light sources

Although light with wavelengths within 600–700 nm is in general the most efficient for photosynthesis, resorting to irradiances between 400 and 500 nm may increase the overall rate of growth. The rationale for this realization lies on the fact that light serves other purposes than photosynthesis only, which are wavelength-dependent: e.g., specific

wavelengths stimulate flowering, stem growth and germination in higher plants. In microalgal cultures, it has been suggested that at least 5–10% photons of blue light are required (if red light were used) for other metabolic functions besides photosynthesis; hence, a small amount of white light may be needed in PBR, in order to account for such non-photosynthetic needs (Kommareddy and Anderson 2004).

Apart from the aforementioned general considerations, the design and selection of efficient light sources for microalgal cultivation depends on the microalga at stake (as different species of microalgae require distinct light spectra, depending on the major pigments present therein), and the type and intensity of the light source—as both spectral quality and quantity of light are important parameters for microalgal growth and metabolism.

Types of light source

The selection criteria of artificial light sources for cultivation of photosynthetic microorganisms include high electrical efficiency, low heat dissipation, good reliability, high durability, long lifetime, reasonable compactness, low cost and spectral output falling within the absorption spectrum of the microorganism of interest (Bertling et al. 2006).

Full-spectrum light—about half of which is photosynthetically useful (i.e., 400–700 nm), is normally used for microalgal growth; however, it has already been recognized that blue (420–450 nm) and red (660–700 nm) light are as efficient for photosynthesis as the full spectrum.

The main characteristics of the different types of light are depicted in Table 2, as well as their relative luminous efficacy, i.e., the amount of light produced (in lumen) for each watt of electricity consumed. In incandescent bulbs, the energy is emitted mostly in the infrared and far infrared region, so it is very different from those ranges required by photosynthesis—and, consequently, it will be rather inefficient. Furthermore, they usually exhibit a spherical shape and irradiate light in all directions—so reflectors are necessary to direct the light back into a PBR, which increases costs.

The design of halogen lamps is essentially an improvement of the technology used in incandescent bulbs, in attempts to improve their energy efficiency. Although the spectrum is very similar to incandescent lamps, the light output level does not diminish over time, as happens in standard incandescent bulbs (Kommareddy and Anderson 2003).

Fluorescent lamps are usually manufactured from long glass tubes, containing mercury vapor at low pressure with a small amount of an inert gas (e.g., argon). Most light is emitted in the visible region (400–700 nm), and is thus closer to the spectrum of daylight (Kommareddy and

Table 2 Photonic features of major types of artificial light

Type of light	Generation of light	Intensity (W/m ²)	Energy emission in the region 400–500 nm	Energy emission in the region 600–700 nm	Conversion of energy into heat	Shape of lamps	Direction of light	Lifetime (typical value)	Cost	Luminous efficacy (lm/W)
Incandescent bulbs	Heating of tungsten-wire in a vacuum or inert gas environment	5.1	0.5%	3.8%	Very high	Spherical	Irradiates in all directions	750–2,000 h degradation of light output along time	Low price	10–18
Halogen lamps	Heating of tightly coiled tungsten filament surrounded by halogen gas	1.6	0.3%	3.3%	High	Spherical	Irradiates in all directions	3,000–4,000 h light output level does not diminish over time	Low price	15–20
Fluorescent lamps	Production of electric arc leading to mercury vapor ionization by exciting outer shell electrons; upon dropping back to their ground state, energy is emitted as radiation. Inner walls of glass tube coated with fluorescent powder of phosphorus, activated by radiation emitted and fluorescing at longer wavelengths	5.9	25.0%	20.7%	Low	Tubular	Irradiates in all directions	10,000 h duration degradation of light output along time	10× more expensive than incandescent bulbs	35–100
Gro-Lux fluorescent lamps	Gas-discharge lamps that use electricity to excite mercury vapor; excited mercury atoms then produce short-wave ultraviolet light that causes phosphor to fluoresce, thus producing visible light	3.7	18.9%	37.9%	Low	Tubular	Irradiates in all directions	15,000 h duration degradation of light output along time	3–10× more expensive than incandescent bulbs	50–70
Light emitting diodes (LEDs)	Semiconductor light source. Upon switching on, electrons are able to recombine with holes within the device, releasing energy in the form of photons	14.7–55.5	0.04–0.08%	87.6–98.3%	Very low (below 10%)	Elliptical	Irradiates in all directions, or collimates light when using proper lens	35,000–50,000 h degradation of light output along time	2–10× more expensive than fluorescent lamps	25–64
Laser diodes	Based on doping of a very thin layer on the surface of a crystal wafer. To produce an n-type region and a p-type region, one above the other, thus resulting in a p-n junction				Negligible	Elliptical	Irradiates in all directions	High quality diode lasers with 100,000 h	2× more expensive than LEDs	30–45

Anderson 2003). A special type of fluorescent lamp has been marketed (Gro-Lux™) that produces more red light, and thus improves photosynthetic efficiency—but it is also more expensive, and presents similar physical limitations as regular cool fluorescent lamps.

Based on data pertaining to the effect of the light spectrum upon the rate of photosynthetic metabolism, one concludes that light sources with narrower spectral outputs that overlap the photosynthetic absorption spectrum are photosynthetically more efficient, while also dissipating less energy as heat. Such a goal can, theoretically, be attained using LEDs—which, in essence, are semiconductor devices. Like any diode, a LED consists of a *p-n* junction, where electron and hole transfer takes place when voltage is applied at the ends of those *p* and *n* regions (Kommareddy and Anderson 2003). The emission spectrum of a red LED lies in the vicinity of 650 nm, so it is particularly efficient for photosynthesis. Furthermore, LEDs are usually coupled with proper lens to collimate their light beam; hence, they may become very directional, and most light emitted may be directed into the PBR.

LEDs can generate light fluxes well in excess of $2,000 \mu\text{mol}_{\text{photon}}\text{m}^{-2} \text{s}^{-1}$, and also be turned on and off very rapidly (ca. 200 ns) (Tennesen et al. 1995). Furthermore, they are typically small and have a half-power bandwidth as narrow as 20–30 nm, so they can be exactly matched to the photosynthetic action spectrum (Lee and Palsson 1996); LEDs with a peak wavelength of 643 nm have been considered as the most cost-efficient light source to operate PBRs, for over 1 year under continuous mode (Kommareddy and Anderson 2003).

The use of LED arrays to illuminate cell well plates, combined with automatic rack systems for easier handling, has been patented (Everett 2002). LEDs with peak emittance of 680 nm (and a half-power band width of 20 nm) were used as sole light source for cultivation of *Chlorella vulgaris*—with fluorescent light being tested for comparative purposes. The narrow-spectrum monochromatic red light was found to reduce the average cell volume from 60 to $30 \mu\text{m}^3$ —although it did not affect total biomass production, as the number of cells produced was double (Lee and Palsson 1996). After they were grown under LED light, the size of the microalgae became normal once they were exposed to fluorescent light. These results suggest that increased productivities can be attained by cultivating microalgal cells under red light, followed by transfer to white light, in order to increase their size and consequently the overall biomass.

In another study, LEDs with different wavelengths were used to grow *Spirulina platensis* under photoautotrophic conditions (Wang et al. 2007): higher light intensities generated more biomass, and the largest specific growth rate occurred with red LEDs—whereas blue LEDs yielded

the lowest biomass production values, probably due to absence of absorption bands of chlorophyll in the wavelengths of the latter.

The most usual method of producing high intensity white-light with LEDs is to resort to a phosphor material that converts monochromatic light from a blue or UV LED to a broad-spectrum white light—in much the same way followed by a fluorescent light bulb. Currently, cold white LEDs are the most efficacious ones since they perform similarly to fluorescent lamps; their high efficiency arises from the very strongly correlated color temperatures (often above 5,000 K) that produce a “cold” bluish light. In addition, their efficiency is not affected by shape and size, and they have a particularly long lifetime (up to 50,000 h).

Finally, a mention is deserved by laser diodes—a light source that efficiently delivers radiant energy within the narrow wavelength ranges effectively used by photosynthetic microorganisms, together with negligible heat dissipation. Bertling et al. (2006) grew the photosynthetic bacterium *Rhodobacter capsulatus* under two different light sources: a vertical-cavity surface-emitting laser (VCSEL) and an incandescent bulb. For a given growth rate in light-limited culture, VCSEL required no more than 30% of the electric power needed by the incandescent bulb. The optical output of a laser diode is focused on a very narrow wavelength band—the center-wavelength of which cannot be adjusted by more than ca. 10 nm; since the red (or infrared) wavelengths that are particularly efficient to grow some types of phototrophs may be inefficient for other types, this waveband feature may actually constitute a disadvantage when that source of light (which is intrinsically expensive) is intended for use with various photosynthetic species. In any case, laser diode technology holds an extra advantage: its ability to conduct light into an optical fiber, where specific sensing probes can be fabricated to monitor selected chemical parameters (Silva et al. 2008; Jesus et al. 2009).

Intensity of light source

The intensity of a light source is a measure of the number of photons available for photosynthesis; the general effect of light intensity upon growth of photosynthetic cells is illustrated in Fig. 3. The four areas depicted correspond to the lag, exponential, stationary and death phases, respectively. Each type of microalga is characterized by a specific curve that correlates its growth rate with incident light intensity, at a given temperature—as these relationships are temperature-dependent (Carvalho and Malcata 2003, Carvalho et al. 2009); the saturation intensity increases typically with temperature (Kommareddy and Anderson 2004).

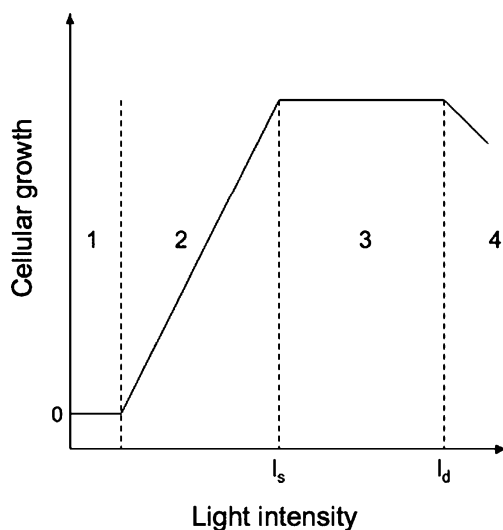


Fig. 3 Typical growth vs. light intensity curve for microalgal cultures (the various symbols are explained in the main text)

Photobioreactor design

One major problem in designing efficient bioreactors for photoautotrophic microorganisms arises from realization that light attenuates exponentially as it penetrates into the culture medium. The average light intensity (I_{av}) at any given point within a culture—assuming that a plate configuration is employed and light comes from a normal direction thereto, can be calculated via

$$I_{av} = \frac{I_0}{\phi_{eq} K_a C_b} [1 - \exp(-\phi_{eq} K_a C_b)] \quad (7)$$

where I_0 is the light intensity on the culture surface, ϕ_{eq} is the length of the light path from the surface to the point of interest in the growth medium (in m), K_a is the extinction coefficient for biomass (usually ca. $0.037 \text{ m}^2 \text{ g}^{-1}$), and C_b is the biomass concentration (in g m^{-3}) (Molina et al. 2001; Kommareddy and Anderson 2002, 2003). Unless the culture exhibits a high ratio of area per volume of the culture vessel, or else a low cell density, light will be available only in a narrow zone close to the culture surface.

In order to overcome such short light penetration depth, the distance between the innermost region of the culture and the light source should be reduced as much as possible—usually via a thin tubular structure, or strong stirring (to increase the efficiency of light usage). However, the former approach leads to inefficient transfer of gases (i.e., supply of CO_2 and removal of O_2), whereas the latter has a maximum limit set by the excessive shear stress imposed on the culture (that may eventually cause cell disruption).

Richmond and Zou (1999) entertained cultures with cell concentrations above $10 \text{ g}_{\text{dry weight}} \text{ L}^{-1}$ in flat plate bioreactor configurations, with narrow light-paths, under vigorous stirring and strong incident light: decreases in the

light-path produced major improvements in photosynthetic productivity, up to optical paths of ca. 1.0 cm.

PBR devices that combine dense microalgal cultures with short light/dark cycles experience enhanced light flux tolerances, and thus improved productivities. Such achievements were obtained only in narrow reactor channels, using flow rates that allow average light/dark cycle times of the order of tens of millisecond, as well as light fluxes up to $8,000 \mu\text{mol}_{\text{photon}} \text{ m}^{-2} \text{ s}^{-1}$ (Gordon and Polle 2007); the productivity recorded reached ca. $17 \text{ g m}^{-2} \text{ h}^{-1}$, which is far higher than the usual figures of $1 \text{ g m}^{-2} \text{ h}^{-1}$. A logical explanation for such an improvement lies on the reduced light path, which shortens the average light exposure to the millisecond level; recall that this value is close to the time scale of the rate-limiting dark reaction of photosynthesis.

Another alternative to increase the ratio of illuminated surface to volume is optical fiber-based PBRs. Mori (1985) reported a system where solar light was collected by fresnel lenses, transmitted through optical fibers and dispersed by ca. 100 light radiators inside the bioreactor. A similar type of reactor had been designed and constructed earlier by Matsunaga et al. (1981), in which light from a metal halide lamp was transmitted and distributed inside the reactor, using ca. 700 light-diffusing optical fibers. These approaches represent relatively nonconventional features in PBR configurations, yet with a number of shortcomings—such as complexity of configuration, difficulty in heat sterilization, and lack of mechanical agitation with consequent risk of cell adhesion to the fiber surfaces.

Optical fibers are usually employed in PBRs as cables to transmit light collected externally in parabolic solar apparatuses. However, direct sunlight (filtered, if convenient) can also be delivered into the bioreactor via suitable optics and large-core optical fiber cables; they can supply the necessary photons to support photosynthesis, by taking advantage of distributors inserted between the vertical membrane tubing where microalgae grow. By controlling attenuation through the fiber optic cables, coupled with especially designed distributor plates made of similar material, a uniform distribution of photons may be assured (Bayless et al. 2006; Xu et al. 2009).

Chen et al. (2006a, b) developed a bioreactor with a ternary light-source system—which combined optical fibers, and halogen and tungsten filament lamps, in order to increase production of H_2 by the photosynthetic bacterium *Rhodospseudomonas palustris*. The PBR consisted of a sealed glass vessel, in which modified optical fibers were inserted from the top; the plastic (polymethyl methacrylate) cladding of conventional end-light optical fibers was removed by mechanical polishing, so as to obtain side-light optical fibers. External light sources were also mounted on both sides of said reactor. When using a single light source, external ones led to higher yields than

optical fibers—probably due to the lower irradiation area of the latter. However, when the three light sources were combined together, under the same total light intensity as when they were used individually, there was a clear enhancement in productivity; this was likely due to the better distribution of light provided by the optical fiber, coupled with supply of light of a wider overall wavelength range.

Fleck-Schneider et al. (2007) modelled growth of, and metabolite synthesis by *Porphyridium purpureum* in a PBR containing optical fibers, used to distribute externally generated light into an internal draft tube—which also served as irradiation element. The surface of the tube was texturized so as to produce a rough structure, able to diffuse light uniformly. Those authors also coupled a shutter module between the light source and the optical fibers, so deliberate light fluctuations could be generated within a wide range of frequencies. The ends of the fibers were ring-shaped, and arranged at the circumference of the glass draft tube; therefore, this tube served both as a confining element for the culture medium and as a transmission element for the light required by the microalgae.

An alternative method to transport light into microalga-containing medium is via light guides that can transmit light deep into the system with negligible intensity loss, and without increasing shear forces as well (Kommareddy and Anderson 2002). Light guides are usually constituted by 9.5 mm-diameter acrylic rods, with either a cone-shaped or a flat end; the rod ends are polished and buffed. Tests carried out revealed that the flat end guides project light forward, whereas the cone-shaped ones project light equally forward and to the cone sides (Anderson 2002). The light transmitted through an acrylic light guide increases significantly with the quality of its surface finish: the smoother (i.e., the more transparent) the finish at the ends of the acrylic rods, the better the light transmission through them, as incident light upon a rough surface will reflect back away from the guide.

PBRs are also often designed to exhibit a high I_{A_0} , so as to work at high biomass concentrations for a given yield, Y_G . Good examples specifically designed to harvest the maximum amount of light are the alveolar panel reactor by Tredici (1999), and the tubular reactors by Richmond et al. (1993), Grima et al. (1994) and Zittelli et al. (1999)—as well as reactors that combine natural and artificial light (Chaumont 1993; Ogbonna et al. 1999). The capacity of optical fibers to conduct light inside a PBR—where it can be dispersed more or less uniformly, can be used either to cultivate photosynthetic microorganisms or to perform light-dependent reactions; this is the case of catalytic photo-reduction of CO_2 to methanol (Wu et al. 2005), or degradation of volatile organic compounds in continuous flow reactors (Danion et al. 2004).

Conclusions

In order to increase microalgal biomass and specific metabolite productivity, long-term strategies of research in the field should encompass design and development of innovative PBRs, coupled with genetic engineering of strains. To attain maximum productivity in said PBRs, several parameters are to be accurately controlled; however, none of the many reactor configurations built to date is able to effectively handle all those parameters simultaneously.

A crucial parameter is surely light supply and harvest; there have been some advances in the use of new or modified light sources, e.g., texturized optical fibers and LEDs with specific wavelengths, but the options available at present are still poor in terms of overall performance. On the other hand, the issue of light fluctuation within a range of frequencies, aimed at short light flashes separated by long dark periods, has not been sufficiently explored in practice—neither have the genetic improvements in reducing the size of chlorophyll antenna molecules, in attempts to enhance photosynthetic efficiency. Therefore, there is still a long way in the field of biophotonics—which will require a more comprehensive elucidation of the light harvesting and utilization pathways and underlying control mechanisms in the cells themselves, coupled with engineering of the light sources and conductors so as to supply the most appropriate light intensity, frequency and spectrum.

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