Review
Flashing LEDs for Microalgal Production

Peter S.C. Schulze,1,2 Rui Guerra,3 Hugo Pereira,2 Lisa M. Schüler,2 and João C.S. Varela2,*

Flashing lights are next-generation tools to mitigate light attenuation and increase the photosynthetic efficiency of microalgal cultivation systems illuminated by light-emitting diodes (LEDs). Optimal flashing light conditions depend on the reaction kinetics and properties of the linear electron transfer chain, energy dissipation, and storage mechanisms of a phototroph. In particular, extremely short and intense light flashes potentially mitigate light attenuation in photobioreactors without impairing photosynthesis. Intelligently controlling flashing light units and selecting electronic components can maximize light emission and energy efficiency. We discuss the biological, physical, and technical properties of flashing lights for algal production. We combine recent findings about photosynthetic pathways, self-shading in photobioreactors, and developments in solid-state technology towards the biotechnological application of LEDs to microalgal production.

Artificial Light in Microalgal Production
Microalgae are a promising biological resource for the mass production of lipids, sugars, polymers, or proteins for the food, feed, and chemical industries [1]. The coproduction of high-value biomolecules such as polyunsaturated fatty acids, carotenoids, β-glucans, and phycobiliproteins for nutraceutical, pharmaceutical, and biomedical applications increases the value of microalgal biomass and the economic feasibility of microalgae-based biorefineries [2]. Presently, high production costs of €5–25 kg−1 hinder the economic feasibility of microalgal-based commodities [3,4]. The European Commission is supporting research and development of microalgal biotechnology by allocating ~€40 million annually between 2007 and 2017 to microalgae-based ventures. For photo- and mixotrophic (see Glossary) microalgal cultivation, light is one of the most important growth parameters; it can come from natural (sun) or artificial (lamps) sources [4,5]. Although artificial light costs more than sunlight, it allows tight control of microalgal biochemistry and growth, increasing the reliability of industrial processes for the production of high-value biomolecules [4,5].

However, the competitiveness of any artificial light-driven microalgal production hinges on energy consumption. Decreased energy costs require improvements in photon harvesting by microalgae and the photon conversion efficiency of light sources. Better light energy usage by phototrophs can be achieved by tailoring species-specific emission spectra of artificial light sources [5,6]. Another strategy concerns not the light quality but light delivery. Instead of using continuous illumination, recent studies propose using flashing lights (Figure 1, Key Figure). Flashing light is intermittent light that can provide highly intense light flashes with a short duration (called the light ‘flash period’ or t) alternating with extended dark periods (td). One flash period followed by a dark period can be defined as a flashing cycle (tc, in which tc = t + td). The use of high light flash intensities (I) enables light to penetrate deeper into the culture and mitigate light attenuation [7–9] in photobioreactors, maintaining high photosynthetic efficiency in

Trends
Light sources require technical fine-tuning for efficient emission of intense light flashes that match the kinetics of the photosynthetic apparatus and are able to penetrate deeper into microalgal cultures. Flashing light may decrease the energy required to achieve a given productivity compared to continuously supplied light. Flashing light systems require higher light output during the light period than standard light sources, and this can be achieved by increasing the current supplied to the LED.

Efficient flashing light emitters are single-color LEDs and laser diodes rather than organic LEDs (OLEDs) or phosphor-converted white (pc-)LEDs. Laser diodes will be promising for future flashing-light sources and may induce beneficial quantum effects on microalgae.
Key Figure

Simplified Diagram of a Microalgal Production Unit Using Flashing (Upper Panel) and Continuous Light (Bottom Panel) Emitted by Light-Emitting Diodes (LEDs)

**Flashing light**

\[
\eta_{\text{WPE}} = \eta_{\text{PWM}} \times \eta_{\text{Ballast}} \times \eta_{\text{LED}}
\]

\[
f = \frac{1}{t_c - t_d} - 1
\]

\[
t_c = t_d + t_i
\]

\[
\phi = t_i \cdot t_i^{-1}
\]

\[
l_i = l_o \cdot \phi^{-1}
\]

**Continuous light**

\[
\eta_{\text{WPE}} = \eta_{\text{Ballast}} \times \eta_{\text{LED}}
\]

**Figure 1.** Flashing or pulsed light can increase light penetration depth and decrease excitation dissipation mechanisms, improving biomass productivity. A flashing light emitting system can transmit tailored wavebands that increase further photon penetration depth (e.g., green light for chlorophytes and Stramenopiles–Alveolata–Rhizaria species) or stimulate metabolic pathways and biochemical composition (e.g., blue and red light). The wall-plug efficiency (\(\eta_{\text{WPE}}\)) is the product of the efficiency factors of all devices between power source and light output, including the efficiencies of the ballast (\(\eta_{\text{Ballast}}\)), pulse-width modulator (\(\eta_{\text{PWM}}\)), and the LED (\(\eta_{\text{LED}}\)). Flashing light devices emit light flashes (\(t_f\)) and dark periods (\(t_d\)) in an approximately rectangular waveform. This waveform is commonly described by the flashing light frequency (\(f\)) and the duty cycle (\(\phi\)). The frequency (in Hertz, Hz) is the number of light–dark intervals (flashing cycles, \(t_c\)) that occur per second (s\(^{-1}\)). The duty cycle is the ratio between the flash period and the whole flashing cycle. The light intensity (in \(\mu\text{mol photons m}^{-2} \text{s}^{-1}\)) during \(t_f\) is defined as flash intensity (\(l_f\)), while during \(t_d\) no light is emitted (e.g., \(l_d = 0 \ \mu\text{mol photons m}^{-2} \text{s}^{-1}\)). Under these conditions the time-averaged light intensity (\(l_o\)) during one flashing cycle can be expressed as \(l_o = \phi \times l_f\) and is used to compare flashing with continuous light treatments. Moreover, the flash intensity and the duty cycle are inversely proportional at a given mean light intensity.

concentrated cultures [10,11]. To prevent photo-damage and inhibition of the phototroph under cultivation by over-intense light flashes, the repetition rate of the light–dark transition (i.e., flashing light frequency, \(f\)) and the relative proportion of the light flash period (i.e., the ‘duty cycle’, \(\phi\)) within the flashing cycle should be adjusted to the biological reaction kinetics of photosynthetic processes and energy dissipation mechanisms (also often referred to as non-
photochemical quenching, NPQ). Nonetheless, well-engineered light sources are essential to emit efficiently flashing light regimes that are advantageous for phototrophic cultivation [4,12]. Balancing these factors, flashing light can result in higher growth performance per input energy than the same light energy supplied in a continuous way [4,12]. We discuss the technobiological threshold for an efficient flashing light system in terms of (i) biological, (ii) physical, and (iii) technical factors that are crucial for applying this promising tool to microalgal cultivation.

Biological Boundaries

Microalgal cultures can display similar or higher photosynthetic rates under flashing light than under continuous light at the same mean light intensity. This is referred to as the ‘flashing light effect’ [13,14] or the ‘light integration effect’ [15] and occurs if the photosynthetic apparatus is working close to its full capacity (a biological factor). However, in cultures with light attenuation, the flashing light effect can additionally be achieved by enhanced light delivery into the culture (a physical factor), even though the photosynthetic apparatus is operating at rates that are far lower than its full capacity. In this section we define the flashing light conditions (e.g., frequency or duty cycle) at which the photosynthetic apparatus perceives a flashing light effect as a biological boundary. Generally, the flashing light effect refers to the response of a phototroph to mean light intensity \( I_d \) during the flashing cycle, and not to the instantaneous light intensity of the light \( I(t) \) or dark \( I_d(t) \) periods (Figure 1). When exposed to frequencies that are too low (e.g., \( f = 1–10 \text{ Hz} \)) with duty cycles that are also too low (e.g., \( \phi > 0.5 \)), no flashing light effect takes place and phototrophs quench excess energy during the light period, and might experience enhanced respiration during the dark period (e.g., post-illumination respiration) [13,15,16]. This results in less growth and biomass losses. Moreover, molecular responses to stress in the phototroph under cultivation can also be activated.

The biological boundary depends on the reaction kinetics of the energy dissipation mechanisms, energy storage, and the linear electron transfer chain (Box 1). Flashing light studies on single leaves of land plants or microalgal cultures with low light attenuation potential can identify the biological boundary, and may be described as a function of frequency \( f \) and duty cycle \( \phi \), in other words \( f_{\phi,\phi} \) [17,18]. For example, Jishi and colleagues [17] identified this flashing light effect for lettuce (Lactuca sativa). Interestingly, their model also fits the photosynthetic performance of microalgal cultures with low biomass concentrations (<0.1 g L\(^{-1}\)) or short light path lengths (<1–2 cm), and of land plants (e.g., tomato) under various flashing light conditions [19–27].

Excitation Dissipation and Energy Storage Efficiencies

Absorbed light energy is able to bring chlorophyll from its ground state (Chl) to a singlet excited state \( ^1\text{Chl}^* \). \(^1\text{Chl}^* \) can pass its excitation energy via resonance or excitation energy transfer to adjacent chlorophyll molecules in the light-harvesting complexes or the reaction centers of photosystem I or II (PSI or PSII). In the reaction centers, charge separation takes place and excitons can be photochemically quenched by provoking the transfer of electrons to the photosynthetic linear or cyclic electron transfer chains [28]. These photosynthetic pathways are essential for the production of ATP and reducing equivalents such as plastocyanin and NADPH [28]. If the reaction centers are ‘closed’, in other words if they are not able to process photon excess under high light conditions, \(^1\text{Chl}^* \) can dissipate absorbed energy as heat through excitation dissipation mechanisms or re-emit a photon (fluorescence) when falling back to its ground state (Chl). Both processes prevent the formation of triplet Chl \( ^3\text{Chl}^* \) which causes the production of reactive oxygen species (ROS) [28]. If, for example, the storage capacity for reducing equivalents cannot cope with the excess electrons under high light intensity, the likelihood of ROS accumulation increases. Such high ROS levels suppress protein synthesis, which is essential for repairing PSII upon photodamage [29]. To avoid excess ROS evolution during high light (flash) periods and maintain their metabolism during prolonged dark

**Glossary**

**Intermittent light**: includes flashing or pulsed light and fluctuating, flickering, or oscillating light. Light and dark periods of flashing or pulsed light conditions shift in an all-or-nothing rectangular waveform. Fluctuating or oscillating light is a fluent transition between high and low light periods, whereas instantaneous light intensities alter continuously over time, usually following a sinusoidal waveform. Sunflashes or cells moving from light to dark zones within a photobioreactor through mixing usually follow fluctuating light patterns.

**Light attenuation**: self-shading by microalgal cells is the most challenging bottleneck limiting the productivity and maximal achievable cell concentration in photobioreactors. Cells located at the periphery of a culture absorb most of the incoming light and may become photoinhibited, whereas cells at the inner layers remain in the dark and become phototolerant. This results in high respiration and energy dissipation rates, causing inefficient photobioreactor use. The depth of the light penetration depends primarily on absorption by cells under cultivation, the incoming light intensity and wavelength, and cell morphology (e.g., cell size) and biochemistry (e.g., pigment contents).

**Mehler and Mehler-like reactions**: these control light-dependent O\(_2\) consumption. Unlike the Mehler reaction, the Mehler-like reaction involves flavodoxin proteins to reduce O\(_2\) without evolution of reactive oxygen species (ROS). Mehler-like reactions enable cyanobacteria, microalgae, and plants to cope efficiently with intermittent light regimes.

**Minimal response times (\( t_{\min} \))**: the minimal response time of LEDs and transistors can be calculated by \( t_{\min} = \phi \times t_f^{-1} \), where \( \phi \) is the duty cycle and \( t_f \) is the flashing frequency at ‘cut-off’ obtained from a frequency response graph (i.e., Bode plot).

**Photosynthetic efficiency**: this refers to how much light (e.g., amount in photons or energy) is required by a phototroph to fixate CO\(_2\) or produce O\(_2\) through photosynthesis (e.g., \( \mu \text{mol} \text{CO}_2 \) or O\(_2\) per \( \mu \text{mol} \) of photons). The effects
periods, phototrophs employ different energy quenching and storage strategies under flashing light with a low duty cycle (e.g., φ < 0.1; Figure 2) [14,16,30,31]. Usually, alternation between light and dark periods longer than seconds, minutes, or hours (implying frequencies < 1 Hz) are referred to light that is supplied intermittently, discontinuously, or through light/dark cycles or photoperiods. For the sake of convenience, however, the term ‘flashing light’ and associated parameters will be used in all timescales.

In this context, mechanisms of short-term energy storage (fs–ps timescale) follow the laws of quantum dynamics, and energy transport takes place via quantum coherence. The excitation energy delivered by light flashes with a duration of fs–ps can be stored in pigment cofactors (e.g., chlorophylls, carotenoids, or phylloquinones) as excitons or through inter-protein hopping within the light-harvesting complexes [32]. If reaction time permits, energy may be stored in reaction center II. In this timescale, excess energy may be dissipated through ultrafast reacting quenchers (e.g., chlorophyll a) [32,33], resulting in pigment internal thermal decay or fluorescence.

Medium-term energy storage (ns–μs timescale) may take place via components and products of the non-cyclic photosynthetic electron transfer chain. Examples are the plastoquinone bound to PSII (Qa–), plastoquinol (PQH2) in the plastoquinone (PQ) pool [34], protons in the stroma coming from the water-splitting reaction catalyzed by the water oxidizing complex, and ATP produced by the ATP synthase in the thylakoid [35]. However, if the previous storage mechanisms are unable to handle excess energy, other non-photochemical quenching reactions and biomolecules seem to play a protective role: for example Mehler-like reactions, the proton gradient regulator PGR5, the ferredoxin-plastoquinone reductase PGR1, the serine/threonine-protein kinase STN7, and several flavodiiron proteins [8,36–38].

For long-term energy storage (ms–s timescale), phototrophs produce reduced equivalents (e. g., NADPH) or ‘high-energy’ chemical bonds via ATP-dependent nitrogen and sulfur assimilation as well as carbon fixation. The last process yields Calvin–Benson cycle intermediates containing ATP-dependent high-energy bonds, such as bisphosphoglycerate or triose phosphate [30]. At this timescale, excess energy can be quenched via reoxidation of the PQ pool through the quinol terminal oxidase, phosphorescence, or through the initiation of diadinoxanthin–diatoxanthin, violaxanthin–astaxanthin–zeaxanthin, or lutein epoxide cycles [39–41].

Energy storage for an even longer term is possible. Biochemical processes at timescales of minutes and hours, such as the accumulation of non-structural low molecular weight sugars, starch, or amino acids (e.g., glutamine as the first amino acid resulting from nitrogen assimilation) can be used to store energy [42]. Under these conditions, excess energy can be quenched through the same photoprotective pigment cycles as under ms–s conditions, but also through high-energy state (qE) quenching and photoinactivation of PSII (here referred to as qI) [33,41,43]. To decrease excess energy that phototrophs receive under longlasting light periods, reversible phosphorylation of the light-harvesting complex II and a decrease in the light-harvesting antenna size might occur [37,39].

Generally, the ratio between non-photochemically and photochemically quenched energy, and the probability of damaging the photosystems by ROS evolution, as a result of a failure of the excitation dissipation mechanisms in place, increases with light flash period duration, causing a drop in photosynthetic efficiency. Under frequencies and duty cycles that are too low and too short, respectively, for obtaining a biological flashing light effect, phototrophs use more complex and energy-demanding excitation dissipation mechanisms during the light period of flashing light on the photosynthetic efficiency of single cells or chloroplasts and whole cultures should be distinct. Dilute cultures with narrow light paths and negligible light attenuation are usually used to identify the effects of flashing light on single cells or chloroplasts (biological boundary). However, flashing light was mostly found to improve photosynthetic efficiency of whole microalgal cultures with high light attenuation (e.g., highly concentrated cultures).

**Photo- and mixotrophy:** phototrophic organisms use light as an energy source to fix inorganic carbon dioxide in organic compounds. Heterotrophic organisms obtain energy and carbon from organic sources (glucose or acetate). A few microtrophic microalgae are able to obtain energy and carbon skeletons by means of photosynthesis, active predation, endocytosis, and membrane-bound transport systems. Some others are even able to steal chloroplasts from other microalgae using a mechanism known as myxocytosis.

**Pulse-width modulation:** a tool used to control the power supply (e.g., dimming) of electrical devices such as LEDs. It generates a pulse wave signal (i.e., rectangular pulse wave) with an asymmetrical shape (i.e., the duration of the on-off cycle) described by the duty cycle.
Box 1. Kinetics of the Linear Electron Transfer Chain (LET)

Photons emitted from a light source are absorbed through light-harvesting pigment complexes within femtoseconds. About 300–500 ps are necessary to transfer shared excited energy states (excitons) through inter-protein hopping and magnetic resonance to the reaction centers of PSI (reaction center II or P680), causing the excitation of an electron. The reaction center requires two electrons for reduction and ‘closure’ (i.e., P680*; Figure 1) [32]. Once the reaction center is in the “closed” state, further excess photon energy cannot be transferred to the reaction center II and is released through energy dissipation mechanisms. The low redox state of the P680* reduces the primary electron acceptor phoepihin within 5–8 ps, becoming oxidized (P680*). The electrons from phoepihin are transferred to the primary (Qo) and secondary (Qi) acceptor sites within ~200–500 ps and 700–1200 μs, respectively. Upon Qi reduction, this site acquires protons from the stroma, forming plastoquinol. In turn, plastoquinol diffuses towards the PQ pool in the thylakoid membrane upon exchange with one PQ molecule, which binds to the Qo site. The PQ pool serves as an energy store. The energy is retrieved upon the oxidation of plastoquinol by the cytochrome b6f complex via the q-cycle [77].

The high redox potential of P680* initiates an electron transfer from the water-oxidizing complex through the intermediate electron carrier tyrosine, which reduces P680* in a succession of steps (S0–S3). Full oxidation of two water molecules and the release of four electrons takes place in about 1–2 ms [35]. As P680 is formed, the reaction center II reopens and the subsequent exciton capture takes place.

The slowest (~3–5 ms) and thus limiting step in the linear electron transfer chain is the oxidation of plastoquinol by cytochrome b6f. Two protons are released into the thylakoid lumen and electrons are transferred towards plastocyanin. Plastocyanin transfers electrons towards PSI within 150–550 μs. In PSI, electrons are passed to the electron donor P700 (reaction center I), forming P700* through photon energy delivered by the light-harvesting complex I within femtoseconds. Electrons are passed to the electron acceptors A0, A1, and the 4Fe–4S iron–sulfur centers Fx, Fa, and Fb within picoseconds, reducing the final electron acceptor, ferredoxin. Because of these short turnover times, the reactions in P700 are considered to be a spontaneous reaction [32,34]. Ferredoxin can donate electrons to ferredoxin-NADP+ reductase to form NADPH, completing the LET. The cyclic electron transfer chain is activated to produce additional ATP and NADPH required for carbon assimilation via the Calvin–Benson cycle.

Figure I. Simplified Diagram of the Major Kinetics of Electron Transfers in Phototrophs, Showing the Linear Electron Transfer Chain (LET) and Other Alternative Pathways. Reaction times and pathways are summarized from [35,39]. Note that the stoichiometric values for H+, ATP, and NADPH are variable. To balance electron flow under fluctuating light regimes, phototrophs use, in a species-dependent manner, different Flv proteins to reduce oxygen to water at the expense of NADPH or electrons from the photosystems [74]. Abbreviations: A1, phyloquinone-based electron acceptor; Cytb6f, cytochrome b6f complex; Cyt b6f, F, b-type hemes cytochrome b6f/F; FA/FB and Fx, electron acceptor 4Fe–4S iron–sulfur centers; FeS, Rieske iron–sulfur protein; FD, ferredoxin; Flv, flavodoxin protein; Flv, Flavodoxin; FNR, ferredoxin-NADP+ reductase; LHC, light-harvesting complex; P680, photosystem II; P700, photosystem I; PC, plastocyanin; Phe, pheophytin; Pi, inorganic phosphorus; PQ, plastoquinone; PQH2, plastoquinol; Qo, primary acceptor site; Qi, secondary acceptor site; Qs, quinone oxidase; RuBP, ribulose-1,5-bisphosphate; Tyr, tyrosine; WOC, water-oxidizing complex.
**Energy storage**
- Pigment cofactors
- Protons in thylakoid lumen, electrons in LETC, CET
- NADPH, biphosphoglycerate, triose phosphate, N, S, and C assimilation
- PQQ, ATP
- Storage lipids
- Storage carbohydrates

**Excitation dissipation mechanisms**
- Thermal decay
- Fluorescence
- RC-quenching
- Thermal quenching through PGR5, PGRL1, FDPs, or STN7 protein
- Phosphorescence
- CET, PSI, and PSII pQ-pool oxidation
- Dd-Dt, VAZ, Lx-L cycle (e.g., diatoms, plants, green algae)
- Q1 (e.g., qE)
- Q2 (e.g., qL)

**Effects on photographs**
- Flashing light effect: Minor intracellular responses
- RuBisCO activation
- Increase of Chl a:b ratio
- LHCII reallocation and aggregation and LHP reduction
- Protective pigment accumulation
- Unsaturated fatty acid and lipid accumulation
- Storage and NPQ capacity

<table>
<thead>
<tr>
<th>fs</th>
<th>ps</th>
<th>ns</th>
<th>μs</th>
<th>ms</th>
<th>s</th>
<th>Ks</th>
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<tbody>
<tr>
<td>Light flash duration (t, in s)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Photosynthetic efficiency</td>
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Figure 2. Response Timescales of Phototrophs Exposed to Different Flash Period Durations (t) with High Flash Intensity (f) That Arise if the Flashing Light Is Composed of a Short Constant Duty Cycle (e.g., φ < 0.1) and a Saturating Mean Light Intensity (f). A given flash period duration is inversely proportional to the flashing light frequency (f). Timescales of events were obtained from results summarized in Tables 1, S1 and elsewhere [33,34,37]. Bar length represents the approximate timescale of the initiation of a given event. Abbreviations: CET, cyclic electron transfer chain; Chl, chlorophyll; Dd-Dt, diadinoxanthin–diatoxanthin; FDPs, flavodiiron proteins; LETC, linear electron transfer chain; LHC, light-harvesting complex; LHP, light-harvesting pigment; LX-L, lutein epoxide; NPQ, non-photochemical quenching; PGR5, proton gradient regulator 5; PGRL1, a ferredoxin-plastoquinone reductase that is apparently involved in CET in chlorophytes; PQ, plastoquinone; PQQ, plastoquinol; PS, photosystem; Q1/2, quenching sites 1 and 2; qE, energy state quenching; qL, photoinactivation of photosystem II; RC, reaction center; RuBisCO, ribulose-1,5-bisphosphate-carboxylase/oxygenase; STN7, a serine/threonine-protein kinase involved in the adaptation to changing light conditions; VAZ, violoxanthin–astaxanthin–zeaxanthin.

(e.g., photoprotective pigment synthesis or high-energy state quenching [33]), and respiration rates exceed photosynthetic rates during the extended dark period [15,44,45]. Both situations will ultimately decrease or restrict net photosynthetic efficiency [27] and alter the biochemical profile and appearance of microalgal and cyanobacterial cells. Changes include cell size, pigment composition, intracellular ultrastructure, the expression of protective proteins (e.g., PGR5 or STN7), the ratio between PSI and PSII [8], light-harvesting antenna size, ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBisCO) activity, and sugar and starch contents [8,11,30,46–51]. These changes are typical for responses of microalgae to intense light [49,50], and thus can be used as indicators if the frequency and duty cycle are inadequate for a phototroph to experience the biological flashing light effect. Conversely, if phototrophs are exposed to increasing frequencies (e.g., f > 10 Hz; φ = -0.1–0.5), these changes become less obvious [46–48,52,53] because a phototroph buffers and quenches photoenergy delivered.
during the light period with a similar efficiency to that under continuous light. Nevertheless, lower intracellular chlorophyll a and carotenoid contents are probably not good indicators for the flashing light effect because lower amounts of these pigments occur in several species under a wide range of flashing light conditions (e.g., \( f = 0.1–100 \text{ Hz} \); Table 1) [44,46–49].

**Table 1. Impact of Flashing Light with Different Duty Cycles and Frequencies on Microalgal Composition**

<table>
<thead>
<tr>
<th>Microalga</th>
<th>Frequencies (( f ))</th>
<th>Duty cycles (( \phi ))</th>
<th>Outcome</th>
<th>Refs</th>
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</thead>
<tbody>
<tr>
<td><em>Chlamydomonas reinhardii</em></td>
<td>1–100 Hz</td>
<td>0.5</td>
<td>Absorption spectra unaffected by flashing light, no obvious shift in carotenoid:chlorophyll ratio in absorption spectra.</td>
<td>[20,21]</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardii</em></td>
<td>0.5–5 Hz</td>
<td>0.5</td>
<td>Fatty acid profile and total lipids were mostly unaffected by flashing light.</td>
<td>[75]</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardii</em></td>
<td>0.00138–1 Hz</td>
<td>0.5</td>
<td>Decreasing chlorophyll a content with increasing frequency (( L_{0} = 220 \mu\text{mol photons m}^{-2} \text{s}^{-1} )). The lowest amount of chlorophyll a,b and carotenoids was under ( f = 1 \text{ Hz} ).</td>
<td>[44]</td>
</tr>
<tr>
<td><em>Chlorella kessleri</em></td>
<td>5 Hz–37 kHz</td>
<td>0.5</td>
<td>Higher intracellular chlorophyll concentrations under flashing light as compared to continuous light.</td>
<td>[11]</td>
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<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>2.5–25 kHz</td>
<td>0.0125–0.125</td>
<td>Immediate sugar accumulation when exposed to saturating light flashes for 18 h. Dark periods lasting only 6 h led in turn to an accumulation of nucleic acids and complete consumption of accumulated sugars. Protein and chlorophyll levels unaffected.</td>
<td>[24]</td>
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<tr>
<td><em>Dunaliella salina</em></td>
<td>0.017–5 Hz</td>
<td>0.4–0.66</td>
<td>Chlorophyll a content was usually lower under flashing light (( L_{0} = 400 \mu\text{mol photons m}^{-2} \text{s}^{-1} )) conditions as compared to continuous light. A ( f = 5 \text{ Hz} ) gave similar results to continuous light. D. salina exposed to flashing light conditions always displayed a lower total lipid content.</td>
<td>[50]</td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em></td>
<td>25–200 Hz</td>
<td>0.17, 0.33, 0.67</td>
<td>Final astaxanthin and biomass concentrations in the medium were higher under flashing light as compared to continuous light. With increasing duty cycle but the same frequency, the final volumetric astaxanthin concentration rose. The use of flashing light lowered energy consumption for astaxanthin production by up to 70%.</td>
<td>[76]</td>
</tr>
<tr>
<td><em>Isochrysis galbana</em></td>
<td>10 kHz</td>
<td>0.5</td>
<td>No effects on total lipid content in <em>i. galbana</em> or cell weight. Fatty acid profile was similar under flashing and continuous light.</td>
<td>[52]</td>
</tr>
<tr>
<td><em>Nannochloropsis oceanica CY2</em></td>
<td>7,8,9 Hz</td>
<td>0.5</td>
<td>No significant differences in EPA content between cells under flashing and continuous light.</td>
<td>[53]</td>
</tr>
<tr>
<td><em>Nannochloropsis salina</em></td>
<td>1–30 Hz</td>
<td>0.1, 0.33</td>
<td>Flashing light had no effect on total lipid content and usually caused a reduced increase of chlorophyll a and carotenoid:chlorophyll ratios (except ( f = 10 \text{ Hz} ), ( \phi = 0.33 )).</td>
<td>[48]</td>
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<tr>
<td><em>Porphyridium purpureum</em></td>
<td>0.17–100 Hz</td>
<td>0.17, 0.5</td>
<td>A frequency of 0.17 Hz increased the intracellular phycoerythrin and chlorophyll a content as compared to either continuous light or 25 Hz and ( \phi = 0.33 ). Bound and free polysaccharides were affected marginally. Production rates of phycoerythrin and free polysaccharides were highest under ( f = 100 \text{ Hz} ), ( \phi = 0.5 ) (( L_{0} = 540 \mu\text{mol photons m}^{-2} \text{s}^{-1} )).</td>
<td>[49]</td>
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<td><em>Scenedesmus bicuspidatus</em></td>
<td>(~100 \text{ Hz})</td>
<td>0.5</td>
<td>Long-term exposure to flashing light did not affect total lipids, proteins, carbohydrates, fatty acids, or amino acids. However, flashing light slightly lowered chlorophyll a and b levels, increased chlorophyll a/b ratios, decreased carotenoid content, and increased the carotenoid:chlorophyll a ratio. RuBisCO initial activity (not activated) and RuBisCO total activity (activated) were significant higher only under flashing light at a moderate irradiance of ( L_{0} = 175 \mu\text{mol photons m}^{-2} \text{s}^{-1} ), whereas low ( L_{0} = 87.5 \mu\text{mol photons m}^{-2} \text{s}^{-1} ) and high ( L_{0} = 350 \mu\text{mol photons m}^{-2} \text{s}^{-1} ) mean light intensities had no effect.</td>
<td>[47]</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td>5, 10, 15 Hz</td>
<td>0.5</td>
<td>Carotenoid:chlorophyll ratio and chlorophyll a content in cells were lower under flashing light. Carbohydrate, lipid, and protein contents were unaffected.</td>
<td>[46]</td>
</tr>
</tbody>
</table>

*Refer to Table S1 for a detailed overview of relevant flashing light studies on microalgae, cyanobacteria, and plants.
*Abbreviations: EPA, eicosapentaenoic acid; \( L_{0} \), mean light intensity during a flashing cycle; \( L_{i} \), light flash intensity; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase.
Limits of Flashing Light on the Electron Transfer Chain

Emerson and Arnold [54] demonstrated that a short light period with an adequate flash intensity can excite all ‘open’ reaction centers, whereas a sufficiently long dark period allows all reaction centers to ‘reopen’ and harvest most of the incoming photons of the next light flash. A later study by Radmer and Kok [55] quantified that a light-harvesting complex containing 400 chlorophyll molecules harvests ~2000 electrons per second under full sunlight, whereas the subsequent carbon fixation reactions are able to process only 100–200 electrons per second. They showed that the photosynthetic apparatus could only use a small portion of incident light under continuous light, while, most of the time, reaction centers are closed and light is non-photochemically quenched.

Recent findings about excitation dissipation mechanisms and the quenching role of the PQ pool may allow other approaches to determine the optimal flashing light settings required for photosynthesis. It has been suggested that the species-dependent storage capacity of the PQ pool for plastoquinol may define the threshold frequency, duty cycle, and the required mean light intensity beyond which the flashing light effect occurs [26,56]. Vejrazka and colleagues [21] and Hüner and colleagues [34] pointed out that, if excess plastoquinol is generated, the plastoquinol:plastoquinone (PQH2:PQ) ratio becomes too high, leading to over-reduction of the PQ pool, which will prevent the reaction center II from reopening [34]. Such over-reduction occurs if transfer rates of photonic energy exceed the kinetics of its use by metabolic pathways that promote growth, including those involved in nitrogen, sulfur, and carbon utilization. Detrimentally, excess energy can lead to photoinhibition and photo-oxidative damage due to ROS evolution. Phototrophs can dissipate this energy by, for example, (i) activating the cyclic electron flow, (ii) phosphorylating and migrating the light-harvesting complex II towards PSI to reinforce the cyclic electron transfer used to oxidize the PQ pool, or (iii) activating the xanthophyll cycle [39]. However, to avoid photoprotective mechanisms and thus inefficient photonic energy usage, light supply should take place in balance with the reaction kinetics of the linear electron transfer chain. Interestingly, a light flash lasting picoseconds is already sufficiently long to excite and close the PSII reaction centers [26]. To restore the ground state of PSII, and thus to reopen the reaction center II, requires the transfer of this charge from the PSII to the PQ pool in the form of plastoquinol [57] and the reduction of PSII through the water-oxidizing complex. To avoid excess of plastoquinol reducing equivalents in the PQ pool, the reduction rate of PQ to plastoquinol at the Q0 site of PSII should be similar to the plastoquinol oxidation rate at the Qp site of the cytochrome b6f complex. However, an imbalance easily arises because the oxidation of one plastoquinol takes longer (~3–5 ms) than does reducing PSII by the water-oxidizing complex (~1–3 ms). To mitigate such imbalances in the linear electron transfer chain, flashing light may be tailored to a flash period duration of a few hundred picoseconds to reduce efficiently reaction center II without triggering excitation dissipation mechanisms, and a dark period of 3–5 ms to allow the timely oxidation of plastoquinol, avoiding over-reduction of the PQ pool.

However, these kinetics would correspond to a duty cycle of only $\Phi = 10^{-8}$ and a frequency of $f = 300\text{–}500$ Hz. Such settings require low switching times, which are problematic to implement with currently available technologies (Box 2). Indeed, frequencies higher than 300 Hz usually resulted in a flashing light effect in most phototrophs if the mean light intensity was near saturation (e.g., $I_0 \geq 100 \mu\text{mol photos s}^{-1} \text{m}^{-2}$; Table S1 in the supplemental information online). However, decreasing the mean light intensity towards sub-saturating levels appears to require higher frequencies to obtain the flashing light effect [26,58]. More specifically, Martín-Girela and coworkers [58] found a CO2 fixation efficiency of 6.2 photons per fixed CO2 molecule, which was beyond theoretical limits (~8 photons CO2$^{-1}$) at a frequency of 10 000 Hz ($\Phi = 0.05$) with a mean light intensity that was only 5% of the photosaturating intensity.
Box 2. Technical Limits of Flashing Light Sources

The efficiency of a flashing light system depends on working and switching losses at transistors, built-in pulse-width modulators, controlling units, and LEDs that interact differently with applied current, frequency, and duty cycle. For example, work losses in transistor switches increase if duty cycle and currents are high, but they are frequency-independent. Switching losses increase with frequency, although they are current-independent [73]. Photon extraction potential from LEDs is higher if forward current is increased (i.e., LED overloading), and if light and dark periods are sufficiently short or long, respectively, to allow sufficient heat dissipation from the LED-chip (Figure 1). In this case, frequency and duty cycle are interdependent.

Overloading is a valuable option for flashing light applications, permitting the operation of an LED under higher currents that exceed nominal levels (e.g., by increasing the supplied voltage), resulting in maximal photon flux during the duty cycle and heat dissipation during the dark period [11,24]. Through overloading, the LED operates at current densities beyond which the ‘droop effect’ occurs. As result, the photon conversion efficiency drops with increasing forward currents, and maximal photon extraction cannot exceed a given threshold [78]. During overloading and a shortened duty cycle, the maximal extractable mean light intensity ($I_p$) per LED decreases, but the maximal applicable instantaneous forward current and extractable flash intensity increases.

Another parameter is the LED minimal response time ($t_{min}$), which is the major cause for electrical losses when emitting flashing light. The delection region as the central internal element of any LED chip creates capacitances that limit the response of single-color LEDs to a few nanoseconds ($t_{min} = \approx 1–50$ ns). Note that organic LEDs display high capacitances while phosphor-converted LEDs have long (electro)luminescence decay times, and this restricts $t_{min}$ to approximately $\geq 1$ μs [79–81]. Standard laser diodes display very low capacitance, allowing $t_{min} < 500$ ps [82]. Overloading and chip size increases these capacitances and thus response times, limiting maximal adjustable frequencies or duty cycles. Lowering the response times of (organic) LEDs is an active field of research aimed at facilitating efficient visible light communication or screens [80,82,83].

Broadband flashing light research at nanosecond scales can use widely available signal generators connected to high power single-color LEDs or laser diodes as a cheap solution (e.g., < $\varepsilon 2000$). For larger-scale systems with high light output, a more robust and cheaper system may be used, based on a slower-responding open source system ($\geq 1$ μs) consisting of an Arduino microcontroller coupled with standard LED luminaries (for examples see [50,84]).

![Figure 1. The Efficiency of LEDs or Laser Diodes under Pulsed Power Supply Can Be Calculated from Frequency Response Graphs (Bode Plots) (A). The cut-off frequency ($f_c$) refers to the maximal adjustable frequency with maximal 50% power losses (commonly referred to as the 3 dB point). From $f_c$, response times and possible adjustable flashing light regimes can be calculated for different light sources (B). If switching regimes are below 1 ns, a shift from the laws of classical to quantum mechanics occurs. Overloading (C) is achieved if the input power ($P_{in,electrical}$) increases due to higher forward currents that exceed nominal levels. Nominal conditions are obtained when LEDs operate under continuous power supply and rated (or nominal) currents at a given temperature. Under a nominal power input ($P_{in,electrical}$), an LED achieves its nominal light output ($P_{out,optical}$) and nominal efficiency (e.g., photon conversion efficiency; PCE = $P_{out,optical}/P_{in,electrical}$) as defined by the manufacturer. LED light output comes at the cost of efficiency after passing a critical current density beyond which the droop effect occurs (dashed line), and usually reaches a peak (maximum) with subsequent decline. All values are examples and may differ among diodes. Abbreviations: pc-LED, phosphor-converted LED; AlGaAs LED, gallium–aluminium–arsenide LED.](image)

On the other hand, it remains to be seen how phototrophs respond if they are exposed to extremely low duty cycles (e.g., $\phi < 10^{-8}$), with a flash intensity $\sim 10^8$-fold higher than the mean light intensity, which corresponds also to a photon penetration depth eightfold higher than that of continuous lighting [7]. Under such a timescale, dissipation of excess energy may only take place via fluorescence or thermal decay, and other more complex and energy-demanding quenching mechanisms (e.g., mediated by PGR5, PGR1, flavodiron proteins, or STN7) cannot be activated in time [37,59]. This may lead to a more efficient light utilization and higher photosynthetic efficiencies. On the other hand, these conditions could also increase flash intensities above a threshold that would instantly cause photodamage. If so, an under-saturating mean light intensity could be sufficient to achieve the same or even enhanced...
photosynthetic rates compared to continuous lighting with a saturating light intensity, leading to lower power consumption of artificial lighting. Notably, flash intensities that are inhibitory if emitted continuously do not inhibit the phototroph if the frequency is high and the duty cycle is sufficiently short (e.g., \( f > 1 \text{ kHz} \), \( \phi < 0.1 \)) for a given mean light intensity \([24–26,60–62]\). For example, Tennessen et al. \([26]\) exposed tomato leaves to photoinhibitory flash intensities \((I_f = 5000 \text{ \mu mol s}^{-1} \text{ m}^{-2})\) at a short duty cycle \((\phi = 0.01)\) and a high frequency \((f = 5000 \text{ kHz})\) without impairing photosynthesis. Nevertheless, Ley and Mauzerall \([63]\) found that flash intensities higher than \(22,000–37,000 \text{ \mu mol photons m}^{-2} \text{ s}^{-1}\) (e.g., \(10^{16} \text{ photons cm}^{-2}\) supplied during light periods of \(450–750 \text{ ns}\)) can indeed cause a decline of oxygen evolution rates in \textit{Chlorella vulgaris} cultures \((t_d = 2 \text{ s})\). These findings indicate that mean light intensity, flash intensity, frequency, and duty cycle are interdependent and must be well balanced to reap the benefits of artificial flashing light-based phototrophic cultivation.

**Physical Boundaries**

The most important physical factor of flashing light is the potential to mitigate light attenuation and increase light delivery in concentrated microalgae cultures \([13,49,61,64,65]\). Current efforts to enhance light delivery include intensive mixing, light path minimization, antenna size reduction, waveband tailoring, or the inclusion of fibers and nanoparticles as waveguides into the photobioreactor \([9,66–69]\). In addition to these approaches, high light intensities can increase photon penetration depth in suspensions as defined by the Beer–Lambert law \([7]\). This law describes a linear increase of light penetration depth into microalgae cultures with exponentially rising light intensity, although the effects of fluorescence or light scattering by different algae are not considered.

Light intensities that are too high cause photoinhibition of microalgae at the periphery of the photobioreactor, an effect that is mitigated by increasing mixing rates. Higher mixing rates minimize the retention time of cells in the high light zone near the walls and in the dark zones in the middle of the reactor. These high mixing rates improve illumination and can provide light–dark cycles that are sufficiently fast to obtain the flashing light effect \([13,14]\). Particularly high productive cultivation systems benefit from high culture concentrations and light intensities \([70]\). However, these conditions require extremely high mixing velocities, resulting in high energy consumption \([44,62,71]\), shearing, cavitation, and pressure changes that impair the physiology and viability of microalgal cells \([70]\). Alternatively, a light source can directly emit flashing light, and this allows the generation of intense light flashes at frequencies and low duty cycles that do not occur in nature or in any culture vessel merely by adjusting the mixing velocity of the growth medium.

Because mitigating light attenuation is one of the main arguments for flashing light-induced growth enhancement \([13,49,61,64,65]\), production systems that operate at high cell concentrations or culturing vessels with long light path lengths are promising targets for flashing light-related power savings. Although this trend has only been observed in a few studies \([11,61,70]\), the true potential of mitigating light attenuation in dense microalgal cultures remains uncertain, particularly at extremely high light flash intensities (e.g., \(I_f > 10,000 \text{ \mu mol photons m}^{-2} \text{ s}^{-1}\)), delivered at low duty cycles (e.g., \(\phi < 0.01\)) and high frequencies (e.g., \(f > 1 \text{ kHz}\)).

**Technical Boundaries**

In artificial light-based microalgal production, light with low and high light periods (e.g., flickering or fluctuating light) is naturally emitted by common gas discharge lamps, whereas flashing light is generated when the light intensity of LEDs is controlled via pulse-width modulation (e.g., Figure 1). The intensity of light emitted by fluorescent lamps and mercury or sodium vapor lamps changes between maximum and minimum values (often referred as ‘flickering light’) at a ballast- and grid-dependent frequency \([47]\). For example, fluorescent lamps driven by a
conventional magnetic or electronic ballast emit flickering light at frequencies of 100–120 Hz and 40–120 kHz, respectively. On the other hand, induction lamps operate at frequencies ranging from hundreds of kHz to tens of MHz [72]. Therefore, it becomes clear that neither induction nor gas discharge lamps should be used as ‘non-flashing’ controls in any flashing light study. In addition, gas discharge lamps are inefficient in terms of photon conversion efficiency if operated at low duty cycles, and may be unsuitable for customized flashing light modulation. Alternatively, amplitude- or pulse width-modulated LEDs can efficiently emit continuous and flashing light, respectively. However, commonly available pulse width-modulated dimmers generate flashing light only between frequencies of 150 and 300 Hz, which may be not sufficient to obtain a flashing light effect if dimmed, although higher frequencies and lower switching times are possible (Box 2).

For microalgal production, a promising flashing LED device may operate at a (sub)saturating mean light intensity, which requires a light flash intensity that increases in inverse proportion to the duty cycle. The emission of high light flash intensities is possible if the stock densities of LEDs in a luminary array are increased, which has additional costs. Alternatively, the number of photons emitted per light emitter can be enhanced under flashing light if the forward current to an LED is increased far beyond the nominal currents used under continuous operating conditions. This so-called ‘overloading’ demands precise switching regimes to extract the highest number of photons with the highest efficiency possible. Considering all losses between power source and light emission of a flashing light system (referred to as wall plug efficiency), most discriminative parameters include (i) response time and photon conversion efficiency of the LED, (ii) operating frequency and duty cycle, and (iii) the efficiency factor of the electronic ballast and pulse-width modulation unit. Generally, the wall plug efficiency of a flashing lighting system decreases with increasing frequency, decreasing duty cycle, and increasing forward current because of working and switching losses at transistors and LEDs [73]. The efficiency drop can be damped if transistors and LEDs display low response times as well as low working and switching losses under the flashing light condition and currents employed. A joint effort between physicists and biologists will thus be necessary to develop efficient flashing light systems that enhance energy use in artificial light-based microalgal production.

**Concluding Remarks and Future Perspectives**

Using flashing lights is a promising strategy to supply photonic energy to phototrophic organisms, thereby increasing biomass productivities and reducing power consumption in artificial light-based production systems. Flashing light can also be applied to established methods employed to improve the photosynthetic performance of microalgal cultures, such as mixing, light guides, or waveband tailoring. The optimal settings of a flashing light regime should correspond to a frequency that is sufficiently high to obtain the same or higher photosynthetic efficiencies than those under continuous light (e.g., $f > 300$–$500$ Hz; biological factor) at the shortest possible duty cycle to obtain the highest possible photon penetration depth (physical factor), but both within the range of adequate power consumption (technical factor). So far, most studies have tested flashing light conditions with low frequencies ($f < 100$ Hz) and relatively high duty cycles ($\phi > 0.1$) to mimic conditions that are present in mixed microalgal cultures. However, data beyond these conditions will be of particular interest for artificial light-based microalgal production. Mainstream flashing light research and industrial application will benefit specifically from inexpensive and technically mature LED modules as light sources.

Nevertheless, current LED technology cannot modulate flashing light in response timescales close to the boundaries imposed by the biological responses, such as light-harvesting events within the range of femto- to picoseconds. A possible solution to this limitation is the use of faster-responding laser diodes (see Outstanding Questions) which could replace common LEDs in cutting-edge research, as well as in future industrial production facilities. Cultures
exposed to sub-nanosecond light flashes might obey the laws of quantum mechanics, which can result in unforeseeable effects on photosynthesis and growth of phototrophs. Research on charge transfer on quantum level, as for example implemented by the EU project H2020-MSCA-QuantumPhotosynth, may shed new light on the limits of photosynthesis and more efficient photon utilization by microalgae.

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