Green microalgae in intermittent light: a meta-analysis assisted by machine learning

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Microalgal biotechnology still needs to alleviate the productivity bottleneck before achieving the full extent of its promises. With this goal in mind, many studies have investigated the impact of light/dark cycles on microalgae growth. In particular, two-time scales of intermittent light have been investigated for operating a culture in a PBR: medium (0.1 to 10 Hz) and high (> 10 Hz) frequencies. However, regardless of the light regime many conflicting results have been reported. This can be attributed to the belonging of the studied strain to different phyla and the variety of experimental designs. In this review, we propose a comparison of the results published for both frequencies. To compare likes to likes, only studies on green microalgae involving low-density cultures in an optically thin photobioreactor have been selected. Collected data were analyzed using machine learning and inferential statistics. First, the choice of the method for monitoring culture photosynthetic activity (growth rate or oxygen concentration) has an effect on the direction of the results. Second, two trends emerge. For medium frequencies, with respect to continuous light, lower duty cycle values (ε < 0.1) hinder photosynthetic activity (p < 0.001), while higher values (0.5 < ε) produce similar results (p = 0.557). In high frequency regime, lowering the cycle time furthers culture performances (+20 to +73 % depending on the monitoring procedure, p < 0.001). In addition, based on the reviewed evidences, recommendations are drawn to avoid a waste of effort in future works: flat panel airlift combined with intermittent lighting should be the reference experimental device for this type of investigations. Furthermore, growth rate monitoring should be preferred to its dissolved gases counterpart.

Microalgae | Light | Intermittent | Frequency | Machine learning
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1. Introduction

Microalgae have been used by men since the dawn of time, initially to meet the nutritional needs of indigenous populations. Later, in the middle of the last century, their production took on trade dimensions (1, 2). Since then, these microorganisms have constantly drawn scholars and engineers attention. At first, for nutritional reasons, then the field of applications gradually opened up to other domains. Nowadays research initiatives can be split into four major fields: biofuel production, high value added molecules production, wastewater treatment and CO_2 sequestration (1, 3–7). Yet several challenges remain to be addressed before microalgae can realize their full potential including strain optimization (8), cultivation (9), harvest (10) and extraction (11). This review is intended to help address the second one through the efficient use of the limiting resource that is light at the culture vessel level (12).

Like any living organism, microalgae growth is affected by a number of abiotic factors, including temperature, salinity, nutrients, gas concentration (CO_2, O_2), and pH. However, because they are photosynthetic organisms, light is often the main factor limiting growth. This is furthered by the fact that light in itself cannot be stored by cells. Consequently, it has to be continuously supplied to the culture (13). Besides, the interaction between light and microalgae is complex and changing over the course of the culture. At the beginning of the culture, cell suspension is translucent and light penetrates the whole culture vessel (referred to as photobioreactor, or PBR, hereinafter), supplying energy to all the cells. Then, as the culture grows, cells absorb more and more light, resulting in shortening the depth of light penetration. Roughly speaking, this results in the appearance of two zones: the photic zone near the lit surface of the vessel, and the aphotic zone corresponding to almost complete darkness in the inner part of the PBR. This highly heterogeneous light field significantly affects microalgae growth. In addition, as they are cultivated under constant agitation, cells are shuttled through the light gradient. Hence, microalgae experience light intermittency on a timescale of few tenths of second to several seconds depending on both the PBR geometry and the mixing rate (14–18). These changes in light patterns are commonly referred to as Light/Dark, or L/D cycles. They can be characterized by three fundamental parameters, namely the incident light intensity hitting the surface of the culture (I_0), the ratio between the light period and the dark period of the cycle (ε), also called duty cycle, and the total cycle time (τ_c). In literature, these L/D cycles have been reported as having considerable effects on microalgae growth (17–19). Positive influence reports have sparked the idea that efficient L/D cycle management could promote culture growth, hence alleviate part of the burden hindering microalgae mass production. Still, in this context, it is necessary to understand the relationship between the periodic light modulations and the resulting variation in photosynthetic efficiency and growth within PBRs as well as its effect on the production of desired molecules.

In the biotechnological literature, three ranges of light intermittency that are liable to affect microgae have been identified: low frequency light modulations, medium frequency light modulations and high frequency light modulations (20). The first range refers to long L/D cy-

cles of several hours or even days, such as the circadian cycle. The mid-frequency range is defined as L/D cycles of frequency generally between 10 Hz and 0.1 Hz. These are intermittent light patterns occuring naturally in PBRs due to the combination of PBR geometry, mixing rate, and microalgal concentration. Finally, the high frequency range refers to the delivery of light pulses at frequencies above 10 Hz. The exploration of this last frequency range originated from the will to understand photosynthesis (21–23). Later, it was used to manipulate photosynthesis aiming at promoting cell growth. Indeed, some of these frequencies echoes the biological ones, leading to a modulation of the associated biological mechanisms (as an example, the reader can refer to this new method of measurement (24)). In this paper only intermittent high- to mid-frequencies illuminations are considered since these are the frequency ranges likely to have a beneficial effect on microalgal performances.

All these considerations are based on photosynthesis, a sophisticated and dynamic process involving a multitude of reactions, each with its own time scale (25, 26). The photosynthetic response generated by this cellular machinery is particularly sensitive to changes in irradiation, quality, and temporality of light (27). Basically, the photochemical energy transduction begins with photoinitiated electron and proton transfer reactions. The resulting stored electrochemical transmembrane potential leads to further chemical transformations producing energy and reducing intermediate molecules. As long as these reactions are in operation, PSII can not effectively exploit other photons, referred to as closed state. From this observation, one can imagine closing all the photosynthetic apparatuses of a culture once by exposing it to a light flash and wait a little while before sending another one. The in-between flashes duration would have to be tailored to maximize process efficiency. Indeed, a too-short time would not allow photosynthetic apparatuses to reopen, leading to wasting energy when applying the next flash. On the contrary, a too-long dark phase would lead to energy intermediates depletion and growth mechanisms underpowering (28). As a result, depending on the L/D cycle frequency, the light integration differs (Figure 1). In the ideal configuration, the photosynthetic chain would continually build up its stock of energy intermediates while minimizing wasted irradiation. This manipulation of photosynthesis would represent optimal use of light energy (29–31).

Light/Dark cycle manipulation can be achieved with two strategies depending on the aim. Industrially, cells are cultivated in optically dense photobioreactors. As aforementioned, they naturally experience intermittent light. The L/D cycles parameters (ε and τ_c) are distributed around the desired value governed by geometry and mixing. Academically, optically thin photobioreactors (short light path, low cell density) are used to ensure that all the cells perceive the desired pattern in the same way. It allows to investigate intermittent light effect with a high level of confidence. Finally, those two strategies are linked. Indeed,

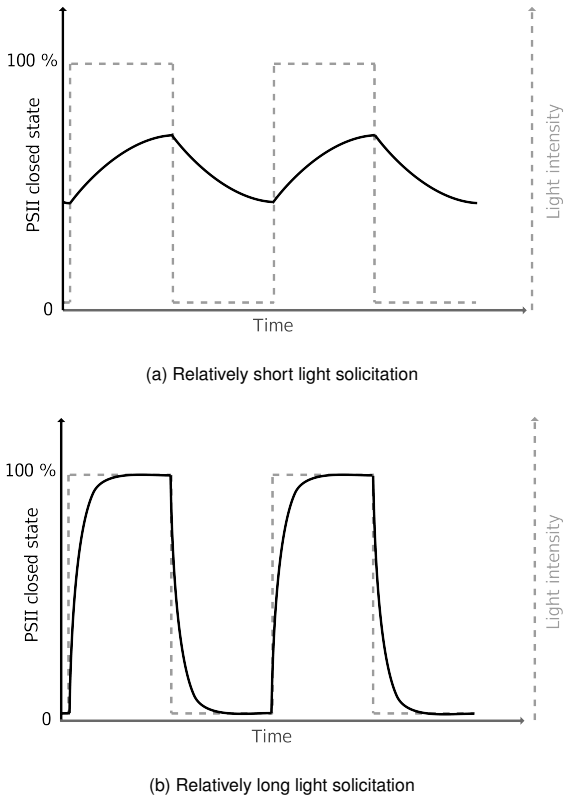


Fig. 1. Schematic representation of different light integration responses by the cells according to the duration of the light loads.

successful scientific investigations would allow to identify promising light patterns (high light use efficiency, low level of NPQ) that would be transferred to industrially relevant cultures (by adjusting geometry and mixing).

Over the years, many studies have been carried out on the impact of L/D cycles variation on microalgae response. These studies were made possible thanks to the pioneering works of Emerson (32), Kok (21) and Myers (23) who established the concepts of photosynthesis and whose outcomes are to be acknowledged. However, since then, various cycles parameters have been applied to cultures and conflicting effects have been reported (16, 17, 20). While some authors have reported stimulating impact on productivity, others have shown no effect or even deleterious effects. The wide variety of microalgal responses to different L/D cycles can be attributed to two main factors. On the one hand, the studies were conducted on microalgal strains belonging to different phyla. However, depending on the strain used, the photosynthetic apparatus structure, and the pigments involved differ and may call upon different response mechanisms (33). On the other hand, the implementation of the experimental design differs from one study to another. Faced with this diversity of responses, there is a clear need to synthesize the results obtained from these studies.

This work aims to decipher the impact of intermittent light fundamental parameters (light intensity, duty cycle, cycle du-

ration) on microalgae performances. We first introduce key concepts about photosynthesis. Then, to provide a high statistical power analysis, we gathered and pooled data from the literature in a systematic manner. Still, to reinforce the relevance of the reported data, we agglomerated results from studies presenting a precise control over parameters of interest. Hence, we focused on studies involving low-density cultures in an optically thin bioreactor. This protocol ensures that all microalgal cells were exposed to the same light pattern within the culture vessel. In addition, to compare likes to likes from a biological point of view, we limited ourselves to studies on green microalgae, specifically on the *Chlorophyta* phylum as it is the most widely used study model but also the most promising in terms of applications (3). The final criterion was the availability of a continuous light reference. After careful reporting, the results are pooled and a meta-analysis is led using both classical descriptive and inferential statistics tools, and machine learning algorithms. Finally, results are discussed from a biological perspective to identify possible avenues for future research.

2. Photosynthesis fundamentals

Photosynthetic organisms such as microalgae perform oxygenic photosynthesis, where light energy is harnessed to create high free energy molecules. This process can be broken down into two stages: light-driven reactions and carbon reactions. During the first one, light energy is absorbed by photosynthetic pigments and channeled through Light Harvesting Complexes (LHC) to the P_{680} reaction centers of photosystem II (PSII) and P_{700} of photosystem I (PSI). This energy drives several biochemical reactions, involving the splitting of a water molecule into oxygen and protons, incidentally releasing electrons. These electrons are used at the end of the electron transport chain to form NADPH (reducing agent). Concomitantly, protons are accumulated within the lumen of the thylakoids. The resulting proton motive force allows the generation of ATP molecules which provide energy to power the carbon reactions along with NADPH (29, 34). This second category of reactions allows the incorporation of inorganic carbon to synthesize carbohydrates used to support cellular metabolism as well as to provide the basic materials of which cells are made.

In the natural environment, microalgae are constantly subjected to variations in light levels, which can become dangerously high. Indeed, excessive energy supply causes photooxidation of PSII components, resulting in photoinhibition (35). This phenomenon ultimately decreases the productivity of microalgae by damaging essential proteins required for electron transfer during photosynthesis. To overcome these deleterious effects, microalgae have acquired feedback and mitigation mechanisms to cope with energy overload and keep electron transport under control (36, 37). These are typically associated with PSII and allow for the dissipation of excess energy through non-photochemical processes. For more information, many reviews are available on the subject, with a particular focus on microalgae (38–45).

3. Reported experimental designs

As aforementioned, the studies selection was based on key criteria: green microalgae as study model, optically fine culture vessel, and the availability of a continuous light reference. Thus, after a thorough search, each potentially relevant study was screened to check whether it met the previous criteria. If retained, the study results were categorized according to the light cycle duration (medium or high frequencies) and the parameter used to monitor photosynthetic activity (cell growth rate (GR) or dissolved gases evolution, P_{O_2} -classically-). The findings are summarized in tables. For the sake of readability, only the findings of the studies with the growth rate as the monitored parameter are presented in Tables 2 and 3. The other results are proposed as supplementary data. First, a synthetic presentation of the key features of the reported studies is proposed. Then, all the data collected are statistically analyzed using Scikit Learn 0.23.2 (46) machine learning tools and analysis of variance tests (ANOVA hereinafter). Finally, they are discussed both statistically and biologically.

3 1. Photobioreactors

In studying the effect of L/D cycle on microalgae, PBR have a key role as they work in synergy with the lighting device (next Section) to ensure proper homogeneous lighting of the culture.

Among the reported PBR designs, a distinction was made between the device for adapting the strain to the light conditions studied and the device used to make the measurements. As a general rule, the device used to measure the growth rate (GR) is the same culture vessel like the one used to acclimatize the strains since it is a medium/long term measurement. However, this distinction is more relevant when the photosynthetic rate (P_{O_2}) is the output variable. Indeed, in these studies, it appeared that the experimental device used for the growth of microalgae can be slightly (17, 47) or even completely different (48–50) from the measuring chamber used to monitor the rate of photosynthesis. This raises the question of strain acclimation before the measurement. Sometimes, it is not possible to conclude as the information was not reported (51).

By taking a closer look only at the cultivation PBR, reported PBR designs can be classified according to their general shape. The most commonly encountered shape is the flat panel PBR (17, 47, 50, 52–56). It usually consists of two wide sheets of glass placed side by side, between which the culture grows. An additional glass sheet can be added to create a cooling jacket and so regulate culture temperature. Bubbling is used to ensure both gas supply and removal as well as mixing. Then come the columns (48, 49, 51, 57) and the tubular (58) reactors. For all those designs, variations exist such as air-lift fluid circulation (48) instead of pumping/stirring or torus shape (30, 59) instead of actual columns shape. Finally, at laboratory scale, flasks

(60) and microwell plates (61) use have been found.

In addition to the choice of a design, the mode of operation has to be selected with care. Two modes of operation are generally employed. On the one hand, classical batch cultures can be undergone (17, 51, 55, 56, 58, 60). Still, as the culture growth induces light gradient within the reactor, cultures are stopped before the cell density becomes too high. On the other hand, semi-continuous (49, 52, 61) or continuous (17, 30, 47, 48, 50, 53, 54, 57, 59) operation can be adopted. In this case, culture is punctually or permanently withdrawn from the PBR and new medium is added. This dilution is usually controlled so that absorbed light (or optical density) is constant, the so-called turbidostat operation.

Finally, when studying light, while the choice of design and its mode of operation are essential to ensure that all the cells have the same light history, it is also crucial to ensure that the PBR is protected from ambient light to prevent disturbance in the measurement. Some studies clearly claim that the PBR was placed in a dark chamber, others make no mention of it, again raising the question of a possible experimental bias.

3 2. Lighting devices

The lighting device is a parameter of equal importance. Although the sunlight is an inexpensive source of light, the data in the reported studies were all subjected to artificial light sources whose spectrum, intensity, and time-variation can easily be controlled. Like the PBR designs, there is a variety of artificial light sources, including incandescent lamps (tungsten lamp, halogen lamp, carbon arc lamp), gas discharge lamps (fluorescent lamp), and Light-Emitting Diode (LED) lamps. Each of these sources has its own spectral characteristics. The choice of the lighting device depends mainly on the desired spectrum and the energy consumption generated by its use. In the selected studies, three light sources were identified: halogen lamps (17, 48, 52), fluorescent lamps (48, 49, 55, 58), and LEDs (30, 47, 49–51, 53–57, 59–61). The first light source produces a blackbody-type spectrum of light, from near-ultraviolet (UV) to deep into the infrared (IR) with the majority of the emitted energy in the infrared and near-infrared regions of the spectrum. The second has radiation comprising visible light as well as a small amount of UV radiation in the UV-A and UV-C ranges. Unlike incandescent and fluorescent lamps, LEDs are capable of emitting narrow continuous spectrum or polychromatic radiation. As a result, different spectrum colors have been identified in the studies. While most of the cultures were conducted under white light (30, 49, 50, 55–57, 59–61), others were carried out under red (47, 51, 53) light or a red-blue light combination (54). Still, for the sake of comparison, light energy cast onto the culture is reported only taking into account the photosynthetically active part of the spectrum (400 to 700 nm).

In addition to the determination of the light source, its application to the cultures has an influence and must therefore be defined. In this regard, two different strategies for studying intermittent light have been used. The first consists of maintaining a continuous light and manipulating cells perceived illuminations using their motion inside of the PBR. For this purpose, opaque elements are placed at regular intervals on the culture device, classically made of a long tube (48, 58). The culture circulating inside will thus be subjected to L/D cycles whose frequency is imposed by the spacing of these darkened bands and/or by the velocity at which the culture circulates. The second approach is to use a lighting system that provides light intermittently on the cultures. Nowadays, LED coupled with fast electronic switches have replaced constant light source combined with rotating sectors. The second type of lighting device is generally used with flat panel PBR.

3 3. Culture performance monitoring

In the design of a study, after choosing the cultivation device and the illumination strategy, the question of monitoring the photosynthetic performance comes. Many parameters can be monitored during the growth of a culture. However, not all of them are relevant. In the context of studies evaluating the effects of a light treatment, two monitoring strategies have been identified. The first is an instantaneous measurement of the cell response by measuring the rate of photosynthesis (P) (17, 47–51). This latter is achieved by the quantification of the carbon fixation rate (CO_2 or O_2 as a proxy for CO_2) per unit of time. Indeed, microalgae incorporate CO_2 to convert it into carbohydrates and they release O_2 as a by-product. The evolution of these gases in the culture medium can be used as an indicator of the cells photosynthetic activity. However, the carbon fixation rate is to be associated with the amount of biomass present in the system. It can be normalized by quantity specific to biomass such as the chlorophyll *a* content, cells number, dry weight or the intracellular carbon (36).

The second monitoring strategy is based on parameters that allow for the assessment of long-term effects of light regime on a culture. In this case, the parameter monitored over time may be optical density, dry weight or cell count (17, 30, 48, 51–61). Afterwards, they are used to compute the culture growth rate.

3 4. Photosynthetic apparatus qualification

In addition to quantifying the effects of a light regime on carbon fixation, some authors also reported photosynthetic apparatus status as well as cells pigments contents. These additional measurements (not shown in tables) aim at enriching the results and ease understanding of the mechanisms at stake during light-culture interaction. For example, when investigating photoprotective mechanisms in *Dunaliella salina*, authors have monitored the evolution of pigment composition and fluorescence. More specially, they followed the VAZ cycle and PSII quantum efficiency, respectively. They showed that this microalga had different

responses when exposed to intermittent and continuous light regimes (62). In particular, cultures exposed to flashing light, 50 Hz, with high incident light intensities, 1000 µmolPhotonsPAR/m^2/s, had a maximum PSII quantum yield approximately 1.2 times higher and a lower respiration rate than the one in continuous light. This suggests that less energy is consumed for cell maintenance. Besides, the carotenoid content was about four times lower than the one in continuous light, indicating a lower level of stress. These cultures exhibited slower zeaxanthin formation kinetics and NPQ induction rates suggesting a slowing down of the photoprotective activity. These changes were accompanied by structural modifications. Indeed, the flashing light-adapted cells accumulated mainly cytoplasmic lipid droplets and few starch grains. In comparison, cultures under continuous light have mainly accumulated starch grains. However, other studies carried out in flashing light at 10 Hz (55) and 100 Hz (63) have shown an unchanged pigment content and biochemical composition with respect to the continuous light reference.

These measurements are interesting since they allow to collect more data on the photoprotection and photoacclimation mechanisms set up by the cell in response to the light pattern (64). However, to date, they are not routinely performed. Therefore, these scarce results do not allow to have a sufficient statistical strength to analyze them in this work.

4. Statistical analysis

Over the course of the literature survey, attention was paid to the experimental device and the culture performance monitoring (GR or P_{O_2}) as well as other key features including strain, L/D cycle parameters, and, when available, statistical assessment (all reported in Tables). Generally speaking, an L/D cycle is defined by different parameters, including the time during which the culture is exposed to incident light (τ_L) of a given intensity (I_0), the time during which the culture is left in the dark (τ_D). From those, the total duration of the cycle (τ_c) is obtained as follows: $\tau_c = \tau_L + \tau_D$. In addition, from these basic parameters, one can deduce the duty cycle (ε) - ratio of the light phase over the cycle duration, expressed as $\varepsilon = \frac{\tau_L}{\tau_c}$, as well as the average light intensity perceived by the cells during a cycle: $I_{avg} = I_0 \times \varepsilon$. In Tables 2 and 3, we report the average light intensity (I_{avg}), the duty cycle (ε), and the total cycle time for readability reasons. The other parameters can be recalculated from the equations given above.

A total of 118 measurement points have been reported for medium frequency intermittent light and 643 for high frequency intermittent light. For all the reported measurement points, the relative variation of the growth rate (or photosynthesis rate) with respect to its continuous illumination counterpart has been computed (η, Eq. 1). A positive value of η represents an improvement over continuous lighting, a negative one a detrimental configuration, and a null one a configuration yielding equal growth. This mathematical treatment aimed at reducing the impact of the specific devices used in each study (PBR type, light source, illumination strategy, ...). Furthermore, in order to compare likes to likes, a weight (study dependent, most of the case ε as incident illumination is often the same between continuous and intermittent light) has been applied so that the amount of light energy supplied is the same between the two quantities $\mu_{\text{Intermitent light, } I_{avg}}$ and $\mu_{\text{Continuous light, } I_{avg}}$. While this correction for the supplied light amount has been used by other authors (50), it is not optimal in the sense that it does not account for growth non-linear dependence upon incident light (65). Still, applying this more elaborate correction procedure would require having had access to a PI curve for each reported study, which was sadly not the case.

$$\eta = \frac{\mu_{\text{Intermitent light, } I_{avg}} - \mu_{\text{Continuous light, } I_{avg}}}{\mu_{\text{Continuous light, } I_{avg}}} \quad \textbf{(1)}$$

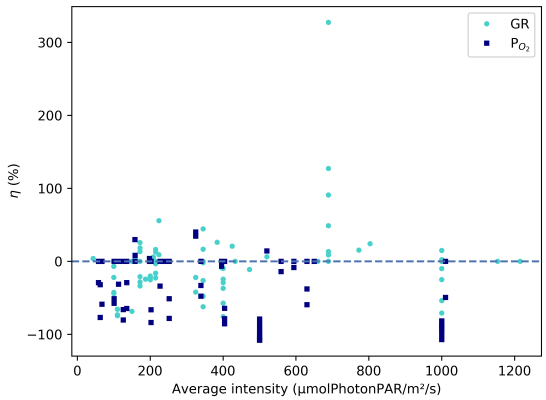
4 1. General trends

Once the values of η have been calculated for each data points, scatter plots were drawn to get a general sense of the dataset. For each light regime, the relative variations are plotted as a function of the different parameters of the L/D cycles, namely the average light intensity (I_{avg}), total cycle time (τ_c), and duty cycle (ε). The results are presented in Figures 2 and 3. As can be seen, the points are widely scattered, still a trend seems to emerge from this crude analysis: low duty cycle values seem to be detrimental to photosynthetic performance in medium frequency regime. Still human capability to explore a 4-dimension space is limited, especially for non clustered data. Thus, classical statistical modeling and machine learning tools were deployed.

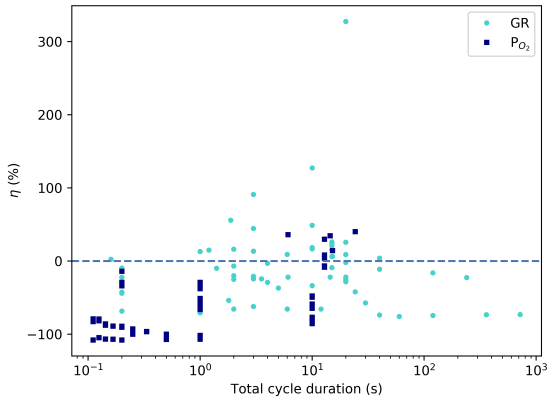
4 2. Machine learning exploration

To explore the dataset, four different machine learning tools were used:

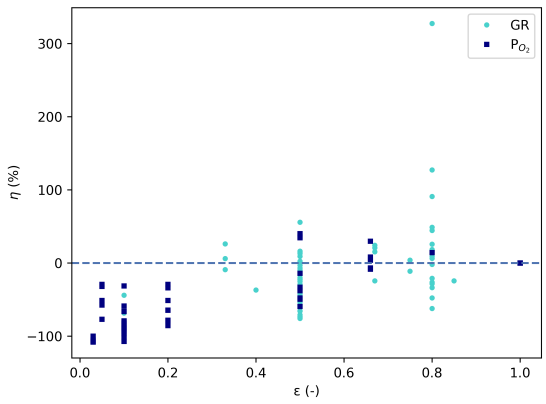
- a simple multilinear model using ε, τ_c (or f) and I_{avg} as predictors of η, fitted using Ordinary Least Square algorithm,
- a non-linear model featuring simple, cross (e.g., εI_{avg}), and square terms (e.g., I_{avg}^2) as predictors of η, fitted using Ordinary Least Square algorithm,
- a LASSO reduction of the non-linear model, in order to reduce model complexity and pinpoint key predictors. In more details, LASSO reduction was based on Least Angle Regression and Akaike Information Criterion (66). This criterion is important as it does not only account for the explained variance but also penalized models using high number of predictors, thus retaining only the most relevant ones,
- a random forest model using ε, τ_c (or f), and I_{avg} as predictors of η. As this is a non-linear stochastic model, robustness was ensured by populating the forest with a high enough amount of trees ('a tree' corresponding to a procedure repetition, 1000 in our case



(a) Light intensity

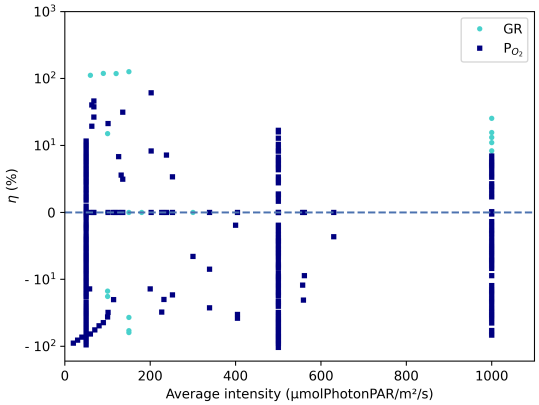


(b) Total cycle time

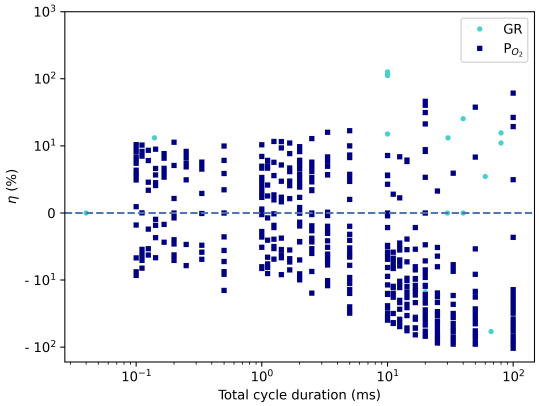


(c) Duty cycle

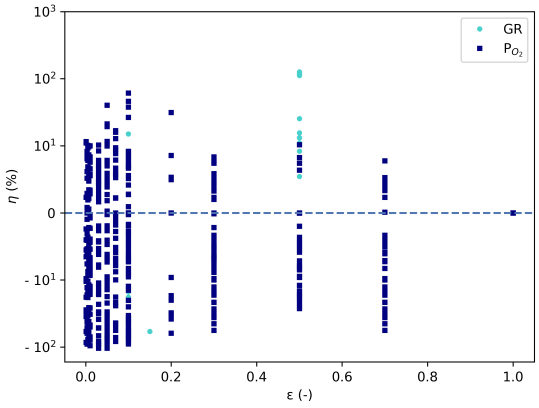
Fig. 2. Graphical representation of relative variations in data from medium frequency intermittent light studies as a function of a selected L/D cycle parameter. Turquoise blue rounds represent data obtained with the growth rate as the monitoring parameter while dark blue squares indicate photosynthetic rate studies



(a) Light intensity



(b) Total cycle time



(c) Duty cycle

Fig. 3. Graphical representation of relative variations in data from high frequency intermittent light studies as a function of a selected L/D cycle parameter. Turquoise blue rounds represent data obtained with the growth rate as the monitoring parameter while dark blue squares indicate photosynthetic rate studies

showed stable results). Furthermore, the model predicts the data using binary choices. The higher the number of choices allowed, the better the prediction. In our case, a maximum number of 6 choices was chosen as it allowed to reach 90 % of the maximum explained variance while limiting overfitting.

Table 1 reports the amounts of variance explained by the different models for the two light regimes. As one can see, for medium frequency regime, the simple linear model is capable of explaining half of the variance, meaning that, as a first approach, low interplay between the predictors can be considered. Adding cross and power terms does not increase the level of explained variance much (from 0.540 to 0.589) while increasing the model complexity. This shows that

higher levels of prediction are not accessible through simple means. This is all the more true as the LASSO algorithm can retain most of the increase in explained variance by adding only one predictor (εI_{avg}, four predictors in total) to the base set. Finally, the random forest algorithm is able to better describe the observed data. Interpreting further random forest insight is not easy because of its inherent randomness, which is a shame because it best represents the reported data. That is why a special tool dedicated to machine learning algorithm explanation was used: the SHapley Additive exPlanations (or SHAP) (67). SHAP graph for medium frequency regime random forest is available in Fig. 4 (a). This graph features three dimensions. First, the predictors are ranked by level of contribution to the explained variance (the highest one explaining the highest amount of variance). In this case, the duty cycle explains more variance than averaged light intensity and cycle duration. Second, the individual observations are placed on the graph for each predictor. Their color indicates the feature value (e.g., blue marker for ε corresponds to a low duty cycle). Third, their location on the x-axis indicates how they influence the computation of the associated η value. Thus, it can be concluded that low duty cycle values contribute to reducing η value, while large duty cycle values favor higher η values, which is in agreement with the previously intuited trend. Regarding averaged light intensity, moderate and high values have a somewhat negative effect on growth while low values are inconclusive. Finally, no clear trend emerges from the analysis for cycle duration.

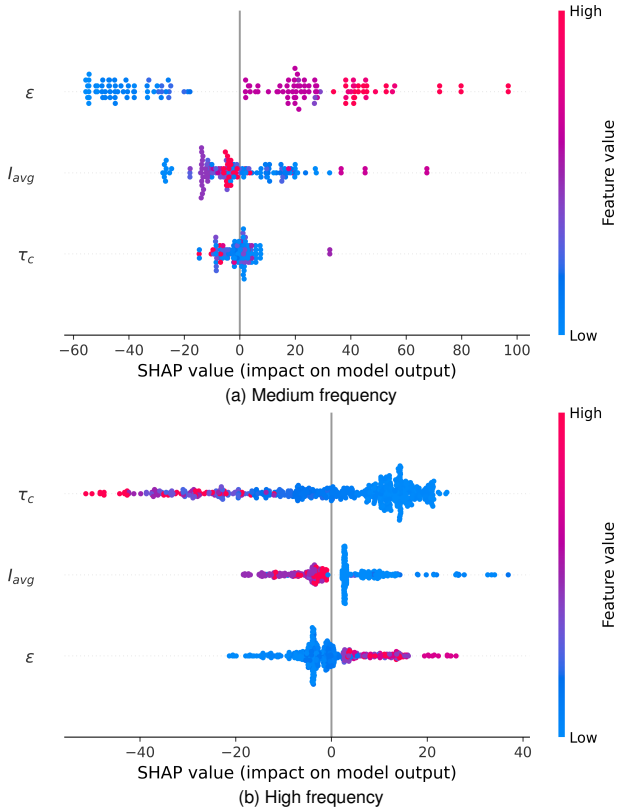


Fig. 4. SHAP graph associated to random forest models for medium and high frequency intermittent light regimes. Predictors: ε , I_{avg} , τ_c, outcome: η. The higher the predictor, the higher the contribution to variance explanation. Color: observation value for the given predictor. x-axis indicates the influence on the η value associated prediction

Model	Explained variance	
	Medium frequency	High frequency
Multilinear	0.540	0.096
Non linear	0.589	0.149
LASSO reduced non linear	0.579 (4/9)	0.149 (8/9)
Random forest	0.808	0.891

Table 1. Variance explained by the four different models for medium and high frequency intermittent light regimes. For LASSO, the number of retained predictors is within parenthesis

The same analysis was undergone for high frequency regime. This time, the multilinear model failed to reproduce the data (explained variance of 0.096). Increasing the complexity of the model by adding additional terms did not increase the amount of explained variance much (0.096 to 0.149). In addition, LASSO reduction had to retain a high number of parameters (8, only εI_{avg} was disregarded). This shows that the data are too complex for a simple model to describe them properly. On the opposite, random forest performed well and managed to explain 89 % of the variance. This highlights the complex non-linear interplay between the predictors at stake. Again, SHAP analysis was carried out and graphed to gain further understanding (Fig. 4 (b)). The most important predictor is the total cycle time, followed by averaged light intensity and duty cycle. While high cycle durations do not exhibit a particular trend (both positive and negative contributions to η), low cycle durations contribute towards higher values of η. Regarding averaged light intensity, low values favors higher growth while medium and high values have an adverse effect. Finally, going from low to high duty cycle values tends to increase the predicted η value.

From this exploration, qualitative conclusions can be drawn regarding the factors influencing the microalgae performance. For the medium frequency regime, a positive correlation has been shown for the duty cycle. This could be explained by the maintenance rate triggered during too-long dark phases (low values of ε). In contrast, a high level of light tends to reduce the value of η, revealing the effect of photoinhibition. In high frequency regime, the trends differ. While short cycle duration promotes η, high light level tends to be unfavorable.

4 3. Inferential statistics

The next step was to use inferential statistics to assess the statistical significance of the observed trends. Indeed, raw data analysis and machine learning exploration pointed out directions. Still, they did not provide quantitative values measuring the level of confidence one can have in the former conclusions (p-values). Thus an ANOVA testing procedure was deployed to quantify this level of confidence. When significant differences ($p < 0.05$) were reported by the ANOVA, a post-hoc Tukey's honestly significant difference test was carried out to pinpoint the discrepancies.

First, we tested the possible impact of an extraneous variable: monitoring strategy (GR or P_{O_2}), a variable that does not describe the L/D cycle *per se*, but was suspected of having an impact on the results. ANOVA procedures reported that there is indeed a significant difference between GR and P_{O_2} monitoring for both medium and high frequency regimes (p-values < 0.001). The potential origins of this and its implications are discussed hereinafter. As a consequence, the data were analyzed separately based on the chosen monitoring strategy.

In order to run those analyses, key parameters values were divided into subcategories. A distinction was made between low ($\varepsilon < 0.1$), medium ($0.1 < \varepsilon < 0.5$), and high ($0.5 < \varepsilon$) duty cycles. The averaged light intensity perceived by the microalgae was divided into low ($I_{avg} <$ 100 µmolPhotonPAR/m^2/s), medium (100 $< I_{avg} <$ 500 µmolPhotonPAR/m^2/s), and high (500 µmolPhotonPAR/m^2/s $< I_{avg}$) intensities. Finally, depending on the light regime, the total cycle time (τ_c) was also subcategorized. In medium frequency, the cycle times were divided between short ($\tau_c <$ 1 s), intermediate (1 s $< \tau_c <$ 60 s), and long (60 s $< \tau_c$) times. In high frequency, the cycle times were split in two as low ($\tau_c >$ 10 ms) and high (10 ms $> \tau_c$).

Looking first at the studies in medium frequency regime (Fig. 5), when GR variation is the studied output parameter, for both medium and long duty cycles (ε), 0 belongs to the 95 % confidence intervals. Thus, it can be stated that duty cycle manipulation does not induce a statistically significant growth performance variation compared to continuous light. On the contrary, a significant difference is observed within the average light intensity category. The intermediate intensities yield lower results ($\bar{\eta}$ = -23 %). While intensities below 100 µmolPhotonPAR/m^2/s and above 500 µmolPhotonPAR/m^2/s are not significantly different from constant light. Finally, for total cycle time (τ_c), while results are globally negative, no significant difference is observed (p-values > 0.05), making further analysis hazardous.

However, the same trends are not observed when the culture performances are monitored using P_{O_2}. Low and medium duty cycles yields similar, negative, performances ($\bar{\eta} \simeq$ -85 %), while high duty cycles reach cell growth similar to continuous light. Low and medium light intensity lead to rather negative performances ($\bar{\eta} \simeq$ -25 %) while high light intensity is statistically lower ($\bar{\eta} \simeq$ -78 %, p-value < 0.001). Total cycle time (τ_c) of less than 1 second and between 1 and 60 seconds give significantly different results (p-value = 0.001, no measurement were made for $\tau_c >$ 60 s with P_{O_2}). Both are still unfavorable to cell growth. All in all, P_{O_2} and GR readings agree on two points: in medium frequency regime, a high duty cycle maintains growth performances and intermediate light yields somewhat lower cell growth ($\bar{\eta} \simeq$ -23 %) compared to continuous light.

If we now consider the studies carried out in high frequency regime (Fig. 6), the trends are dramatically different from those observed for studies in medium frequency. This time, the two culture monitoring strategies yield different results depending on the categories studied. Starting with GR, the three cycle parameters each exhibited significant differences in-between subcategories. Duty cycle between 0.1 and 0.5 is significantly different ($\bar{\eta}$ = -29 %, p-value < 0.05) from the $\varepsilon > 0.5$ group which provides enhanced growth ($\bar{\eta}$ = +40 %, no measurements were made for $\varepsilon <$ 0.1 with GR). Regarding light intensity, averaged intensities below 100 µmolPhotonPAR/m^2/s exhibit significantly better results ($\bar{\eta}$ = +114 %) than those of medium and high intensities which are similar to continuous light (p-values = 0.031 and p-values = 0.027, respectively). Finally, total cycle times higher than 10 ms gave different results than those lower than 10 ms (p-value = 0.001), the latter improving growth ($\bar{\eta}$ = +63 %) while the first yielded performances comparable to continuous light.

Moving on to P_{O_2} monitoring, duty cycle modulation appears to have no effect (p-values > 0.05). Low and medium intensity does not seem to affect growth compared to continuous light or are too underpowered to call. High intensity hinders growth ($\bar{\eta}$ = -19 %, p-value = 0.028). The two cycle duration subcategories gave definitely different results (p-values of 0.001). As for GR, decreasing cycle duration increased growth, but in different amount (from $\bar{\eta}$ = -30 % to $\bar{\eta}$ = -1 %). In the aggregate, for high frequency regime, P_{O_2} and GR readings agree on the trend that decreasing cycle duration improves performance. Still, the other tendencies are to be taken with care since they are not confirmed by both monitoring protocols.

GR and P_{O_2} monitorings report conflicting findings most of the time. This raises the question of the cells' acclimation before entering P_{O_2} measurement procedure. Still, statistical analysis validates machine learning findings. Two points emerge with certainty: for medium frequency regime, high duty cycle allows to maintain growth performances while low values hinder them; for high frequency regime, short cycle duration promotes growth compared to long cycle duration. However, the vast majority of the subcategories exhibit overlapping confidence intervals and point out different directions when analyzing them further. From this analysis, it becomes obvious that no clear conclusion can be drawn and that further research is required.

5. Discussion and guidelines for future research

5 1. Biological mechanisms at stake

From literature, a combination of biological mechanisms are put forward to explain the different effects of intermittent light on microalgae growth. Unsurprisingly, the implementation of these mechanisms differs between microalgae species (50). In general, two situations are distinguished:

Variable category	Sym.	$\overline{\eta_{GR}}$ (%)	$\overline{\eta_{P_{o_2}}}$ (%)	$\bar{\eta}\pm$ 95% CI (%)	Tukey's HSD	p_{GR}	$p_{P_{o_2}}$
$\epsilon < 0.1$	†	-	-89.05		† − ♢	-	0.557
$0.1 < \epsilon < 0.5$	♢	-12.02	-81.65		♢ − ✣	0.900	0.001
$0.5 < \epsilon$	✣	-9.34	-2.48		✣ − †	-	0.001
$I_{avg} < 100$	†	-5.20	-28.06		† − ♢	0.881	0.707
$100 < I_{avg} < 500$	♢	-22.77	-23.11		♢ − ✣	0.001	0.001
$500 < I_{avg}$	✣	34.70	-77.99		✣ − †	0.574	0.076
$\tau_c < 1s$	†	-23.19	-88.56		† − ♢	0.715	0.001
$1s < \tau_c < 60s$	♢	-3.51	-42.68		♢ − ✣	0.083	-
$60s < \tau_c$	✣	-55.88	-		✣ − †	0.596	-

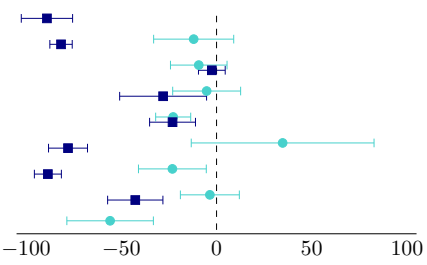


Fig. 5. Medium frequency intermittent light: mean and 95 % confidence interval of the percentage variation of the results of the studies with respect to their reference in continuous light (CL). Turquoise blue rounds markers: growth rate (GR), dark blue square marker: photosynthesis rate (P_{O_2}). Tukey's HSD stand for Tukey's Honestly Significant Difference post-hoc test

Variable category	Sym.	$\overline{\eta_{GR}}$ (%)	$\overline{\eta_{P_{o_2}}}$ (%)	$\bar{\eta}\pm$ 95% CI (%)	Tukey's HSD	p_{GR}	$p_{P_{o_2}}$
$\epsilon < 0.1$	†	-	-12.81		† − ♢	-	0.456
$0.1 < \epsilon < 0.5$	♢	-28.75	-15.66		♢ − ✣	0.008	0.406
$0.5 < \epsilon$	✣	39.60	-9.06		✣ − †	-	0.117
$I_{avg} < 100$	†	114.3	-8.38		† − ♢	0.031	0.847
$100 < I_{avg} < 500$	♢	8.75	-5.69		♢ − ✣	0.900	0.028
$500 < I_{avg}$	✣	8.32	-19.01		✣ − †	0.027	0.847
$\tau_c > 10ms$	†	-10.23	-30.08		† − ♢	0.001	0.001
$\tau_c < 10ms$	♢	63.08	-0.84		-	-	-

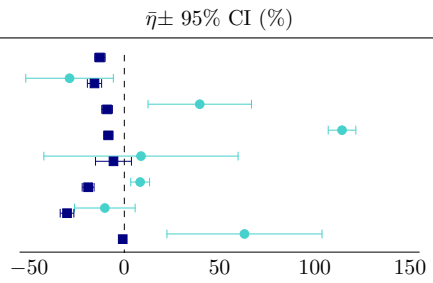


Fig. 6. High frequency intermittent light: mean and 95 % confidence interval of the percentage variation of the results of the studies with respect to their reference in continuous light (CL). Turquoise blue rounds markers: growth rate (GR), dark blue square marker: photosynthesis rate (P_{O_2}). Tukey's HSD stand for Tukey's Honestly Significant Difference post-hoc test

In the favorable case, the photosynthetic machinery under intermittent light works with the same efficiency as, or somewhat higher than, in steady-state under continuous light. The achievement of equivalent efficiency in continuous light is explained by a combination of different factors involving the electron transport chain, the respiration rate and photoprotection mechanisms. In this situation, the amount of photon energy delivered during the light phase is sufficient to support light-driven reactions of the photosynthetic machinery, e.g. reducing PQ pool. The dark intervals have several key roles. At the energy metabolisms level, they must be long enough to allow regeneration of the energy vector stock (PQ pool) depleted during the light phase while being short enough to prevent triggering cell respiration (51, 58). Under such conditions, the overall respiration rate is assumed to be low. This leads to lower energy consumption for cell maintenance allowing energy to be redirected toward anabolic processes (31, 68). At the photosynthetic apparatus level, these dark intervals help to reduce the occurrence of photodamage. When light is emitted punctually, the exposure time to intense light is reduced. These short light exposure periods do not allow enough time for the complete activation of the VAZ cycle and NPQ mechanisms (58, 62). As a result, high intensity light is less dissipated and therefore used more efficiently. Finally, the introduction of periodic intervals of darkness facilitates repair of damage (31, 69). As a consequence, the combination of all these mechanisms ensures a continuous operation of the cellular machinery and allows efficient use of light promoting photosynthesis (54, 62). By analyzing studies individually, best high frenquency intermittent light performances are usually obtained for cycle frequencies between 10 and 100 Hz. This corresponds to 10 to 100 ms, which is the characteristics time for PQ pool oxidation (68).

The unfavorable situation corresponds to the growth of microalgae hindered by intermittent light. This reduction in photosynthetic efficiency is partly explained by an electron transport chain imbalance (12, 51, 70). Indeed, in this case, the light load timescales do not correspond to the reaction kinetics of the linear electron transport chain. Consequently, an imbalance easily occurs, particularly at the level of PQ oxidation, which is the slowest reaction of the photosynthetic chain (12, 58). The latter disturbs the functioning of the photosynthetic chain and becomes deleterious for the cell. For example, casting excessive light on cells with a fully reduced PQ pool does not promote growth. On the contrary, high light is responsible for the generation of reactive oxygen intermediates as well as the proteolytic elimination of protein D1 at the PSII level, inducing photoinhibition. In this respect, an increase in photoprotection and respiration mechanisms have been reported in literature (58).

In both situations, the functioning of the electron transport chain of photosynthesis seems to be at the heart of the mechanism, and more precisely, the maintenance of an energy vector stock (PQ pool). Sending flashes of saturated light allows this pool to be reduced. Its regeneration is dependent on cytochrome $b_{6/f}$, itself dependent on PSI. However, the latter only works in the presence of light. Thus light intermittence has to be tailored so that they can work in synergy. One way to overcome the imbalance is the introduction of a continuous low light background (68). To date, very few studies have investigated this idea. However, the first results obtained are very encouraging.

Improvements in photosynthetic efficiency over continuous light up to more than 50% have been reported for frequencies below 100 Hz (60, 71).

On the contrary to high frequency intermittent light, medium frequency regime mixed findings are much simpler to explain. Individual analysis of the studies shows that performance is usually obtained for cycle durations between 2 and 20 s. This corresponds to a duration too short to induce extensive photoinhibition and too short for dark reactions to depleted ATP and NADPH storages entirely.

5 2. Guidelines

Based on the evidence provided by this literature review, recommendations can be drawn to avoid a waste of effort in the coming research in this field. Here are the proposed guidelines.

First of all, during studies on light-microalgae interaction, the first step is the choice of the experimental device. This step is of paramount importance as it determines the success of the experiment. We have seen earlier multiple PBR designs are available. However, only one makes sense for this type of study. It is the flat panel PBR coupled with intermittent lighting. Indeed, this design guarantees a short optical path. Combining low optical thickness through low cell density, ensures that the illumination is homogeneous throughout the culture. In addition, its flat shape allows to avoid as much as possible undesirable optical effects. For example, the circular shape of a tubular PBR induces a lens effect that leads to heterogeneous lighting inside of the culture medium. Apart from the optical aspect, the gas supply is not limiting as it can be in tubular PBRs. Indeed, as a photoautotrophic organism, microalgae are relatively sensitive to the accessibility of the carbon source. In flat PBR, the gas supply is provided by sparging the gas from the bottom to the entire culture. In tubular PBRs, the formation of a dissolved gas gradient is observed along the long tube. This may lead to both CO_2 depletion and oxygen build-up which affect the culture. In this respect, one study, in particular, investigated the impact of the carbon source availability on the culture (58). Under the same intermittent light conditions but a different CO_2 gas input (10% and 0.03%), the culture gave considerably different results. When subjected to 10% CO_2, the culture had a growth rate twice as high as the one subjected to 0.03% CO_2 underlining the importance of gas supply for the culture. This discussion is summarized in Fig. 7.

The choice of the lighting device is also important. First, the culture device should be set in a place shaded first external light sources. Then, an alternating light source should be used to ensure that all cells are exposed to the same light regime. Indeed, alternating L/D cycles by circulating the culture between areas lit by continuous light and physically darkened areas does not guarantee the same light history for all cells. Indeed, the light perceived by

Photobioreactor type	Possible turbidostat operation	Avoiding gas bluid-up	Avoiding light artefacts
Flat panel	✓	✓ (if not too high)	✓
Bubble column	✓	✓ (if not too high)	✗
Tubular	✓	✓ (if not too long)	✗
Flask	✗	✓ (with specific device)	✗
Microwell	✗	✗ (difficult to keep axenic)	✗ (meniscus effects)

Fig. 7. Suggested criteria to guide the choice of a PBR for the investigation of intermittent light effects on microalgae and their evaluation for different designs

the microalgae is dependent on their trajectory within the PBR. For example, in tubular PBRs, Poiseuille-Hagen's law induces a distribution of velocity inside the tube. The cells at the center of the tube travel faster than those near the walls. This creates a distribution of L/D cycles within the culture.

Properly designing the culture device by carefully selecting a PBR design and appropriate lighting is necessary when investigating light regimes on microalgae. However, this only makes sense if the measurement method used is reliable. Basically, there are two types of commonly used measurements: dissolved gas photosynthesis rate measurements and growth rate measurements. The first one offers a specific and fast method (72). However, the protocols for quantifying the oxygen evolution rate described in the literature are varied and the data collected show significant discrepancies. This phenomenon is highlighted in the Tables 2 and 3. Since these are sensitive measurements, there is a real need to standardize the protocol. Moreover, in addition to the standard precautions to be taken, other questions emerge such as the acclimation state of the cells to the light regime under investigation. The latter remains a central question that has been the subject of several studies (54, 73). On this subject, it has been shown that acclimation of cells to a light condition determined the response of the culture to that same light regime (55). On the one hand, the cells no longer had a lag phase after inoculation. On the other hand, the growth of microalgae was much higher compared to the corresponding unacclimated cells. In addition, acclimated cells showed an increase in the efficiency of light use of approximately 14% compared to non-acclimated cells. The second method of investigation makes it possible to free oneself from the question of the acclimation of the cells. Indeed, acclimation is a cellular process taking place on a time scale from minutes to hours (36, 74, 75). Thus, although growth rate measurements take longer to perform, they ensure that the culture is acclimated to the light regime to be studied. In addition, these measurements have the advantage of closely resembling the operating conditions used in photobioreactors (72) and thus be of high relevance for engineers. However, when leading cell acclimation, a special care has to be taken in managing biofilm (especially for very long turbidostat cultures). There are two reasons for this. First, the biofilm absorbs incident light and modifies the light perceived by the cells inside of the vessel. Furthermore, cells coming from biofilm may exhibit different phenotypic types than the one of interest (suspended cells), hence introducing a bias in the measurement.

6. Conclusions

The influence of light regimes on green microalgae growth was reviewed. The carefully retained studies were subjected to an in-depth analysis of the results employing machine learning and inferential statistics. This investigation showed that higher duty cycle values allow to maintain growth performances while low values hinder them in medium frequency regimes. Futhermore, short cycle duration further culture performances in high frequency regimes. While other findings are also reported, there is a need for further research in order to assert them with certainty. In addition, this review spotlighted that the method for monitoring culture performance has an effect on the direction of the results. Finally, in order to ease comparison between future studies and avoid unnecessary doubt on their results, guidelines are issued regarding both the experimental device and culture performance monitoring. A thin flat panel airlift combined with intermittent lighting should be the reference case. Growth rate monitoring should be preferred when doubt on the cells acclimation arises.

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Declaration of author contributions

WL and VP initiated and planned the project. VP led the statistical analysis. WL drafted the manuscript. PP and VP performed critical revision of the manuscript. All the authors read and approved the final manuscript and take responsibility for the integrity of the work as a whole, from inception to finished article.

Conflicts of interest

Authors have no conflict of interest to disclose.

Statement of Informed Consent, Human/Animal Rights

No conflicts, informed consent, human or animal rights applicable.

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Table 2. All data collected from studies conducted in medium frequency with the growth rate (GR) as the output variable. The table lists the study microorganism, the experimental device used to measure the GR, the parameters of the L/D cycles as well as the experimental results with their coefficient of variation if known (N.A. if not available). The growth rate presented is weighted by the quantity of light. The reference to continuous light appears as CL

Studied microalga	Experimental device	I_{avg} (µmolE/m²/s)	τ_c (s)	ε (-)	Weighted GR (h^{-1})	Experimental CV (%)	η (%)	References
Chlamydomonas reinhardtii CC 1690 wild type 21 gr mt +	PBR design: rectangular PBR (70 mL working volume) Optical light path: 3 cm Light source: halogen lamp Illumination protocol: culture illuminated with a 16/8 h day-night cycle. During the 16 h period, the cells are exposed to different L/D cycles Cultivation mode: batch (final OD_{680nm} <0.25) Measurement: OD difference after 24 h for 2 or 3 days (after 3 days of acclimation in a turbidostat)	650	CL	1	0.116	N.A.	-	(17)
		325	6.1	0.5	0.090	N.A.	-22	
		325	14.5	0.5	0.090	N.A.	-22	
		325	24.3	0.5	0.067	N.A.	-42	
		520	15.2	0.8	0.123	N.A.	6	
Chlamydomonas reinhardtii CC 1690 wild type 21 gr mt +	PBR design: rectangular PBR (525 mL working volume) Optical light path: 1.45 cm Light source: halogen lamp Illumination protocol: culture illuminated with a 16/8 h day-night cycle. During the 16 h period, the cells are exposed to different L/D cycles Cultivation mode: semi-continuous (0.15 <OD_{680nm} <0.30) Measurement: OD difference for 24 h after 2 to 3 days of cultivation	1153	CL	1	0.110	<10 %	-	(52)
		773	15	0.67	0.127	<10 %	15	
		397	15	0.33	0.100	<10 %	-9	
Chlamydomonas reinhardtii wild type strain coded 21 gr	PBR design: glass air-lift loop PBR (0.6 L working volume) Light source: fluorescent light tubes Illumination protocol: PBR placed in a closed cabinet. The dark period obtained with a part of the PBR covered with aluminum foil Cultivation mode: turbidostat (set point: 70% of the maximal flux without algae) Measurement: dilution rate for 66 days	240	CL	1	0.160	>10 %	-	(48)
		158	12.9	0.66	0.167	>10 %	4.3	
Chlamydomonas reinhardtii WT strain CC125	PBR design: microwell plates (150 µL working volume) Optical light path: 5 mm Light source: white LEDs Illumination protocol: cells gradually acclimated to light intensity enclosed in a chamber Cultivation mode: semi-continuous (OD_{750nm} <0.1) Measurement: 3-hourly OD measurements	172	CL	1	0.092	<10 %	-	(61)
		172	3	0.8	0.073	<10 %	-21	
		172	10	0.8	0.061	<10 %	-34	
		172	20	0.8	0.066	<10 %	-28	
		345	CL	1	0.067	<10 %	-	
		345	3	0.8	0.097	<10 %	44	
		345	10	0.8	0.078	<10 %	17	
		345	20	0.8	0.066	<10 %	-2	
		689	CL	1	0.112	>10 %	-	
		689	3	0.8	0.126	<10 %	13	
		689	10	0.8	0.166	>10 %	49	
		689	20	0.8	0.122	<10 %	9	
Chlamydomonas reinhardtii coded 137 AH	PBR design: torus PBR considered as flat panel (1.5 L working volume)	110	CL	1	0.032	N.A.	-	(59)
	Optical light path: 4 cm Light source: white LEDs Cultivation mode: turbidostat (full light absorption and no dark zone)	110	1	0.5	0.011	N.A.	-66	(30)
		110	2	0.5	0.011	N.A.	-66	
		110	6	0.5	0.011	N.A.	-66	

Table 2 continued from previous page

	Measurement: dilution rate	110	12	0.5	0.011	N.A.	-66	
		110	40	0.5	0.008	N.A.	-74	
		110	120	0.5	0.008	N.A.	-74	
		110	360	0.5	0.009	N.A.	-73	
		110	720	0.5	0.009	N.A.	-73	
		200	CL	1	0.041	N.A.	-	(59)
		187	3.53	0.85	0.031	N.A.	-24	(30)
Chlamydomonas reinhardtii	PBR design: flat panel PBR (200 mL working volume) Light source: LEDs Illumination protocol: culture enclosed in a black box Cultivation mode: batch mode with cell concentration always lower than 1.2 g/L at the end of the culture Measurement: OD difference using a correlation with dry weight	100	CL	1	0.073	N.A.	-	(56)
		100	0.2	0.5	0.042	N.A.	-43	
		100	2	0.5	0.068	N.A.	-7	
		100	20	0.5	0.057	N.A.	-22	
		200	CL	1	0.095	N.A.	-	
		200	0.2	0.5	0.074	N.A.	-22	
		200	2	0.5	0.076	N.A.	-20	
		200	20	0.5	0.071	N.A.	-25	
Chlamydomonas reinhardtii CC-124 wild type mt-137c	PBR design: flat panel PBR (360 mL working volume) Optical light path: 25 mm Light source: blue-red LEDs (450 and 630 nm) Cultivation mode: turbidostat (light transmission kept at 60 %) Measurement: biomass concentration difference for 6 h intervals after 72 h of steady state	100	CL	1	0.072	10 %	-	(54)
		100	0.2	0.1	0.040	>10 %	-44	
Chlorella sorokiniana (CCAP 211/8k)	PBR design: glass bubble PBR (380 mL working volume) Light source: halogen lamp Cultivation mode: turbidostat (set point : 70% of the maximal flux without algae) for 1 or 2 weeks Measurement: dilution rate over 3 - 5 days	630	CL	1	0.270	N.A.	-	(48)
		473	40	0.75	0.240	N.A.	-11	
		58	CL	1	0.077	N.A.	-	
		44	40	0.75	0.080	N.A.	4	
Chlorella sp. 11_H5	PBR design: microwell plates (150 µL working volume) Optical light path: 5 mm Light source: white LEDs Illumination protocol: cells gradually acclimated to light intensity enclosed in a chamber Cultivation mode: semi-continuous (OD_{750nm} <0.1) Measurement: 3-hourly OD measurements	172	CL	1	0.074	>10 %	-	(61)
		172	3	0.8	0.084	>10 %	13	
		172	10	0.8	0.088	>10 %	18	
		172	20	0.8	0.093	>10 %	26	
		345	CL	1	0.128	<10 %	-	
		345	3	0.8	0.048	<10 %	-62	
		345	10	0.8	0.067	<10 %	-48	
		345	20	0.8	0.094	<10 %	-26	
		689	CL	1	0.027	<10 %	-	
		689	3	0.8	0.052	<10 %	91	
		689	10	0.8	0.062	<10 %	127	
		689	20	0.8	0.117	>10 %	327	
Chlorella vulgaris SAG 12A	PBR design: flasks (250 mL working volume) Light source: white LEDs Illumination protocol: culture enclosed in a black box Cultivation mode: batch (final cell concentration <1 g/L) Measurement: dry weight after 1 week of cultivation	215	CL	1	0.062	<10 %	-	(60)
		215	1	0.5	0.070	<10 %	13	
		215	2	0.5	0.072	<10 %	16	
		215	4	0.5	0.060	<10 %	-3	
		215	120	0.5	0.052	10 %	-16	
		215	240	0.5	0.048	10 %	-23	
Chlorella vulgaris	PBR design: column PBR (30 mL working volume)	1000	CL	1	0.040	N.A.	-	(51)

Table 2 continued from previous page

	Optical light path: 1.8 cm Light source: red LEDs (645 nm) Cultivation mode: batch (culture diluted <20 µM chl a) Measurement: dry weight increase in the exponential phase for 4 h	1000 1000 1000	1.2 1.4 2	0.5 0.5 0.5	0.046 0.036 0.030	N.A. N.A. N.A.	15 -10 -25	
Dunaliella salina CCAP 18/19	PBR design: cylindrical PBR (500 mL working volume) Optical light path: 7.7 cm Light source: white LEDs Illumination protocol: PBR enclosed in a chamber Cultivation mode: turbidostat (2x10^5 culture <3x10^5 cells/mL) Measurement: cell count difference at 24 h intervals in the exponential phase	400 400 400 400 400 400 400	CL 0.2 4 30 60 3 5	1 0.5 0.5 0.5 0.5 0.67 0.4	0.0429 0.0388 0.0304 0.0183 0.0104 0.0325 0.0271	<10 % <10 % <10 % <10 % >10 % <10 % <10 %	- -10 -29 -57 -76 -24 -37	(57)
Dunaliella tertiolecta CCAP 19/6B	PBR design: rectangular PBR (1 L working volume) Optical light path: 3 cm Light source: halogen lamp Illumination protocol: culture illuminated with a 16/8 h day-night cycle. During the 16 h period, the cells are exposed to different L/D cycles Cultivation mode: semi-continuous (0.15 <OD_{680nm} <0.30) Measurement: OD difference for 24 h after 2 to 3 days of cultivation	660 425 209 1215 803 384	CL 15 15 CL 15 15	1 0.67 0.33 1 0.67 0.33	0.0440 0.0531 0.0467 0.0389 0.0484 0.0491	<10 % <10 % <10 % <10 % <10 % <10 %	- 21 6 - 24 26	(52)
Dunaliella tertiolecta CCAP 19/6B	PBR design: flat PBR (1 L working volume) Optical light path: 3 cm Light source: red LEDs Illumination protocol: culture illuminated with a 16/8 h day-night cycle. During the 16 h period, the cells are exposed to different L/D cycles Cultivation mode: turbidostat (culture <30 mg protein/L) Measurement: dilution rate for 3-5 days after 3 to 4 days of acclimation	433 224 224	CL 6 1.9	1 0.5 0.5	0.077 0.084 0.120	<10 % <10 % <10 %	- 9 56	(53)
Scenedesmus obliquus SAG 276.7	PBR design: flat PBR (150 mL working volume) Optical light path: 1.2 cm Light source: LEDs (CL under fluorescent lamp) Cultivation mode: batch (initial OD_{750nm} = 0.2) Measurement: exponential phase	150 150	CL 0.2	1 0.1	0.036 0.011	>10 % >10 %	- -68	(55)
Scenedesmus quadricauda	PBR design: column PBR (30 mL working volume) Optical light path: 1.8 cm Light source: red LEDs (654 nm) Cultivation mode: batch (culture diluted <20 µM chl a) Measurement: dry weight increase in the exponential phase for 4 h	1000 1000 1000 1000	CL 0.16 1 1.8	1 0.5 0.5 0.5	0.041 0.042 0.012 0.019	N.A. N.A. N.A. N.A.	- 2 -71 -54	(51)

Table 3. All data collected from studies conducted in high frequency with the growth rate (GR) as the output variable. The table lists the study microorganism, the experimental device used to measure the GR, the parameters of the L/D cycles as well as the experimental results with their coefficient of variation if known (N.A. if not available). The growth rate presented is weighted by the quantity of light. The reference to continuous light appears as CL

Studied microalga	Experimental device	I_{avg} (µmolE/m²/s)	Frequency (Hz)	ε	Weighted GR (h^{-1})	Experimental CV (%)	η (%)	References
Chlamydomonas reinhardtii CC-124 wild type mt-137c	PBR design: flat PBR (360 mL working volume) Optical light path: 25 mm	100	CL	1	0.072	10 %	-	(54)
	Light source: blue-red LEDs (450 and 630 nm)	100	10	0.1	0.059	10 %	-18	
	Cultivation mode: turbidostat (light transmission kept at 60 %)	100	50	0.1	0.062	<10 %	-14	
	Measurement: biomass concentration difference for 6 h intervals after 72 h of steady state	100	100	0.1	0.082	<10 %	14	
Chlorella pyrenoidosa	PBR design: horizontal tubular PBR	120	CL	1	0.006	N.A.	-	(58)
	Optical light path: 5 mm	60	100	0.5	0.013	N.A.	111	
	Light source: fluorescent lamps	180	CL	1	0.008	N.A.	-	
	Illumination protocol: black paint periodically shaded tubing	90	100	0.5	0.018	N.A.	118	
	Cultivation mode: batch (final dry weight <1 g/L)	240	CL	1	0.010	N.A.	-	
	Measurement: dry weight variation within a period	120	100	0.5	0.021	N.A.	117	
		300	CL	1	0.009	N.A.	-	
		150	100	0.5	0.020	N.A.	125	
Chlorella vulgaris	PBR design: column PBR (30 mL working volume)	1000	CL	1	0.040	N.A.	-	(51)
	Optical light path: 1.8 cm	1000	12.5	0.5	0.044	N.A.	10	
	Light source: red LEDs (654 nm)	1000	16.7	0.5	0.041	N.A.	3	
	Cultivation mode: batch (culture diluted <20 µM chl a)	1000	25	0.5	0.040	N.A.	0	
	Measurement: dry weight increase in the exponential phase for 4 h	1000	33.3	0.5	0.040	N.A.	0	
Scenedesmus obliquus SAG 276.7	PBR design: flat PBR (150 mL working volume) Optical light path: 1.2 cm	150	CL	1	0.036	>10 %	-	(55)
	Light source: LEDs (CL under fluorescent lamp)	150	10	0.1	0.014	>10 %	-61	
	Cultivation mode: batch (initial OD750nm = 0.2)	150	15	0.15	0.015	>10 %	-58	
	Measurement: exponential phase	150	10	0.1	0.023	>10 %	-36	
Scenedesmus quadricauda	PBR design: column PBR (30 mL working volume)	1000	CL	1	0.041	N.A.	-	(51)

Optical light path: 1.8 cm	1000	25000	0.5	0.041	N.A.	0
Light source: red LEDs (654 nm)	1000	10000	0.5	0.044	N.A.	7
Cultivation mode: batch (culture diluted <20 μM chl a)	1000	7143	0.5	0.046	N.A.	12
Measurement: dry weight increase in the exponential phase for 4 h	1000	33	0.5	0.046	N.A.	12
	1000	25	0.5	0.051	N.A.	24
	1000	12.5	0.5	0.047	N.A.	15