

Microalgal photosynthesis under flashing light

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Chapter 1

General introduction

Microalgae have the potential to produce a great variety of natural products such as: nutraceuticals, fine chemicals, lipids, proteins, starch, etc. Therefore, microalgal products are a promising feedstock for a biobased economy. Large-scale, algae production can be done in open ponds or closed systems (photobioreactors). A photobioreactor (PBR) has the advantage that culture conditions can be controlled accurately and algae productivity is higher than in open ponds.

Productive algae cultures are characterized by full light attenuation along the light path. In other words, light impinging on the surface of algae cultures is completely absorbed along the light path, which results in a light gradient and possibly a dark zone. The length of the dark zone depends on algae concentration, algae pigmentation, light path and photon flux density (PFD) at the surface.

Algae cultures are mixed to assure a sufficient mass transfer and to keep the algae homogeneously suspended. Mixing results in a movement of the algae through the light gradient and the dark zone. The consequence of this movement is an exposure to fluctuating light (movement through light gradient) and light/dark cycles (alternation between light zone and dark zone, Figure 1.1).

At the surface of a PBR or algae production pond individual algae will be exposed to over-saturating sunlight PFDs, whereas the PBR interior can be dark. Darkness is characterized by PFDs below the compensation point of photosynthesis, i.e. where gross photosynthesis matches dark respiration.

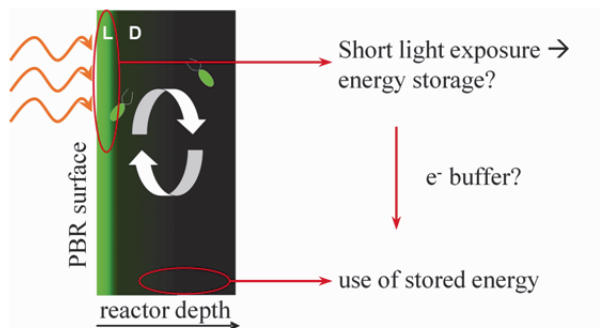


Figure 1.1 Schematic view of light attenuation (L) and a dark zone (D) in a one-side illuminated PBR.

If it is assumed that algae photosynthesis responds to local PFDs then the local photosynthetic rate can be calculated for each layer in the PBR system. The photosynthetic rate depends only on the local PFD, which can be described by a saturation function. The local photosynthetic rate can be converted to a local specific growth rate and the overall growth rate can be determined by integrating the local growth rates over the whole light path or PBR depth.

In this scenario, long exposure to darkness will result in respiration of internally accumulated storage compounds (e.g. triose sugars) and that will lead to a reduced biomass yield on light energy and reduced PBR productivity. Exposure to low PFDs ($< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) will also result in a low biomass yield because a high fraction of absorbed light that has to be used for maintenance purposes. In addition, exposure to over-saturating PFDs at the PBR surface will result in a low biomass yield, because the light absorption rate is much greater than its maximal utilization rate and excess absorbed light energy is dissipated as heat. Therefore, only a narrow range of PFDs ($\approx 100 \mu\text{mol m}^{-2} \text{s}^{-1} - 200 \mu\text{mol m}^{-2} \text{s}^{-1}$) will lead to optimal algae growth and ensure maximal light use efficiency of the PBR system.

In this scenario, photobioreactor productivity can be maximized by eliminating the dark zone (no zone with solely dark respiration) and adjusting the biomass concentration so that the last layer of the PBR system receives just enough light for net photosynthesis.

This response implies that the rate of mixing (and light/dark cycling) does not influence the photosynthetic efficiency which can be reached. The optimal biomass concentration and volumetric productivity will decrease with increase in depth. But, the areal productivity and overall photosynthetic efficiency of the whole PBR system will remain constant. However, from practice it is known that deep and poorly mixed systems result in a lower areal productivity than more shallow and well-mixed systems (Norsker et al., 2011, supplement 1).

The approach based on local rates is valid for a range of different PBRs with depths in the order of several centimeters (Cornet and Dussap, 2009; Takache et al., 2010). In these PBRs cycle times of cells moving from the PBR interior to the light-exposed surface and back are in the order of seconds. Cycle times in raceway ponds will be in the range of 10 s – 100 s. These conditions lead to a further reduction in productivity. In addition, lab-scale experiments showed that the photosynthetic efficiency decreases under light/dark (L/D) cycles in the order of seconds to tens of seconds (Barbosa et al., 2003; Grobbelaar et al., 1996; Janssen et al., 2000).

In contrast to long cycle times in algae production ponds or PBRs with a light path in the range of several centimeters (> 5 cm), very short cycle times can be achieved in short light path (< 5 cm) PBRs if intensively mixed. Cycle times can reach tenths of a second and the photosynthetic efficiency can be higher than predicted based on local photosynthetic rates. Under these short cycle times a phenomenon called 'light integration' has been observed (Phillips and Myers, 1954; Richmond, 2000; Terry 1986).

Light integration is based on the assumption that algae do not respond to the fluctuating light but experience a constant PFD equal to the average PFD (PFD_{av}) along the optical path. Therefore, the over-saturating PFD impinging on the PBR surface is integrated along the optical path to a sub-saturating PFD. A condition for light integration is fast mixing in combination with a very dense culture (dark zone). As a consequence of light integration, the rate of photosynthesis and the specific growth rate are constant over the whole PBR volume and depend solely on the spatial average PFD (PFD_{av}). In this case algae will use light very efficiently because they experience optimal PFDs. One hypothesis to explain the effect of light integration is that algae can store a certain amount of reducing equivalents or electrons, which are produced in the light part. This pool can be used in short dark periods to aid carbon fixation and consequently growth (Figure 1.1).

Full light integration would be ideal for microalgae mass cultures but probably it can only be partially reached in well-mixed and optically thin PBRs. Dedicated lab-scale experiments show that full light integration is observed under fast light/dark cycles of 14 Hz to 24 Hz, or more, provided that the dark period is substantially longer than the light period to reach a low time-averaged PFD (Kok, 1956; Matthijs et al., 1996; Nedbal et al., 1996; Phillips and Myers, 1954; Vejrazka et al., 2011). The highest frequency measured in a PBR was 25 Hz (Perner-Nochta and Posten, 2007) but usually frequencies are in the range of 1 Hz to 10 Hz (Luo et al., 2003; Moberg et al., 2011). Therefore, it is interesting to study sub-second L/D cycles from 1 Hz (1 s) to 100 Hz (0.01 s), whereas the faster L/D cycles (> 25 Hz) are a reference to determine at which point full light integration can be achieved.

Partial light integration can occur in short light path PBRs under sub-second (< 1 s) L/D cycles but the biological response to these L/D cycles is not fully understood. And, there is still a lack of detailed insight in the dynamic response of microalgae and a need for quantitative and predictive models. For a PBR system with known cycle times, these models will help to determine the optimal size of a dark zone to maximize productivity

and photosynthetic efficiency. Furthermore, PBR design can be optimized if the best configuration of L/D cycles and mixing frequencies is known.

The overall aim of this thesis was to investigate the effect of sub-second (1 s - 0.01 s) light/dark cycles on algal growth and photosynthesis. The experimental data was used to develop a mechanistic model. This model describes algae response to the dynamic light regimes.

To complete the aim of this thesis the following sub-tasks were performed:

1. Review existing lab-scale L/D cycle experiments, give a theoretical background of L/D cycles and discuss possibilities to improve PBR productivity by applying specific L/D cycles. **(Chapter 2)**
2. Determine biomass yield on light energy and acclimation state of algae for a selected set of flashing light regimes (minimized light attenuation) in a continuously-operated PBR. **(Chapter 3)**
3. Determine the influence of different duty cycles on oxygen yield on absorbed light energy and photosynthetic oxygen evolution. **(Chapter 4)**
4. Develop and validate a mechanistic model that describes net photosynthetic oxygen evolution under flashing light based on biomass specific light absorption rate and light dissipation rate of excess absorbed light. **(Chapter 5)**
5. Determine the combined effect of fluctuating light (light attenuation) and L/D cycles (dark zone) on biomass yield on light energy in a continuously-operated PBR. **(Chapter 6)**
6. Discuss the implications of L/D cycles on PBR design and operation, and the potential of the developed model **(Chapter 7)**

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Chapter 2

Flashing light effects in photobioreactors

Abstract

Microalgae are promising organisms for a biobased economy as a sustainable source of food, feed and fuel. High-density algae production could become cost effective in closed photobioreactors (PBR). Therefore, design and optimization of closed PBRs is a topic of ongoing research in both academic and industrial environment.

Mixing in dense algae cultures causes light/dark (L/D) cycles of different magnitudes exposing algae to flashing light. It is often said that due to a flashing light effect, productivity of a PBR can be increased. This review will give a theoretical background of the flashing light effect and discuss possibilities to improve PBR productivity by its application.

Photobioreactor performance can be optimized by maximizing photosynthetic rate and biomass yield on light energy based on controlled mixing and L/D cycling. It is unlikely to achieve maximal enhancement based on L/D cycles because of the fast mixing required: specific growth rate measurements in well-controlled, lab-scale PBRs suggest a minimal flash frequency of 14 Hz -24 Hz combined with short flash times (< 20 ms) to achieve a maximal enhancement.

The application of flashing light alone in an artificially illuminated PBR has a limited effect on PBR performance, consequently continuous (sun) light should be preferred. Further optimization strategies can be developed based on mechanistic models that describe the influence of L/D cycles on algae productivity.

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Introduction

Microalgae are very promising organisms for a biobased economy as a sustainable source of food, feed and fuel. Their production does not compete with food production because cultivation does neither depend on fresh water nor on arable land. Microalgae produce a wide variety of natural compounds: products with high market values and bulk chemicals with a low market value like lipids for biofuels (Rosenberg et al., 2008).

Compounds with high market values can be produced in small-scale, indoor photobioreactors with artificial illumination, but production of low-value, bulk chemicals relies on cheap, efficient, large-scale outdoor cultivation systems. Current photobioreactor (PBR) designs are not optimal or scalable, and algae growth and product formation is difficult to predict and to control. Design and optimization of new closed PBRs is, therefore, a topic of ongoing research in both academic and industrial environment (Greenwell and Laurens, 2010; Posten, 2009; Wijffels and Barbosa, 2010).

Algae absorb light that enters the PBR; as a result a light gradient exists between the illuminated surface and the inside of the PBR (Figure 2.2, left column). Mixing will move the algae from illuminated regions at the PBR surface to dark regions inside. Algae experience, therefore, fluctuating light conditions. If the fluctuations in light are fast enough then algae are subject to a flashing light effect, which can result in an increased productivity compared to illumination with continuous light of the same intensity (Degen et al., 2001; Gordon and Polle, 2007; Hu et al., 1998). However, fast mixing requires more energy and it is not clear at which mixing rates increased PBR productivity will be observed. In this review we will give the theoretical background of the flashing light effect and discuss the possibilities to improve productivity in PBRs by means of this phenomenon.

Effects of light gradients in photobioreactors

The energy source for microalgae is (sun) light. Light energy is measured as a photon flux density (PFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$) describing the amount of photons received per unit of area and time. Usually photons with a wavelength between 400 and 700 nm are measured because only these are absorbed and used by algae. Therefore, this wavelength range is called photosynthetic active radiation (PAR). Unlike chemical energy sources (glucose, methanol, etc.) used in other microbiological processes, light cannot be 'dissolved' in the culture medium. Light can only be supplied to the PBR surface as a continuous flux of photons. Either the photons are absorbed by algae at the surface or will pass un-absorbed and penetrate deeper into the PBR, which is called light

attenuation. As a result of light attenuation and mixing, algae receive continuously changing PFDs and could even experience complete darkness while moving through the PBR.

Light attenuation depends on pigment composition of the algae and their concentration (Figure 2.1a). In the scenario in Figure 2.1a, about half of a 3 cm deep panel PBR is in darkness at a biomass concentration of 1 g/L; a further increase in biomass density leads to an illuminated zone of only a few millimeters, a steeper light gradient, and a larger dark zone (Figure 2.1a). This non-homogenous distribution of light energy over the PBR volume makes it necessary to know the dynamic response of algae to an alternating exposure of different PFDs.

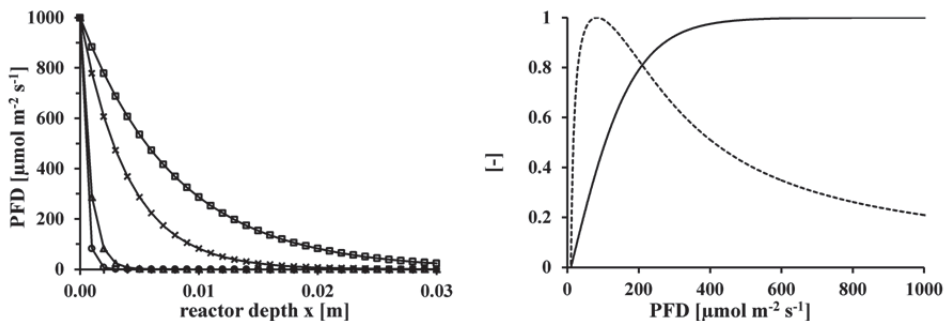


Figure 2.1 a) photon flux density versus photobioreactor depth for four different biomass concentrations: \square 0.5 g L⁻¹, \times 1 g L⁻¹, Δ 5 g L⁻¹, \circ 10 g L⁻¹, light gradient was calculated based on an average dry weight specific absorption coefficient of 0.25 m² g⁻¹ (low light acclimated algae) and Beer-Lamberts' law, b) normalized net photosynthetic rate (—) and normalized photosynthetic efficiency (biomass yield on light energy ---) versus incident photon flux density

The photosynthetic response to different light regimes is typically measured in low density algal cultures where light attenuation is minimized and, consequently, all algae receive a similar PFD. The photosynthetic response can be measured as photosynthetic rates (i.e. biomass specific growth rate, μ ; biomass specific oxygen evolution rate, P_{O_2}), or as biomass yield on light energy ($Y_{x/ph}$). The biomass yield on light is defined as amount of dry matter produced by the absorption of 1 mol of PAR photons and, therefore, it is a measure of photosynthetic efficiency.

The specific photosynthetic rate under continuous illumination is a function of the incident PFD (Figure 2.1b, solid line). At very low PFDs algae experience darkness and the net rate of photosynthesis is negative; more energy is consumed by respiration for cellular maintenance than is produced by photosynthesis. The PFD where net photosynthesis equals zero is called compensation point. At this point the respiration rate

(oxygen consumption) equals exactly the gross photosynthetic rate (oxygen production). The biomass yield on light energy is zero at the compensation point and, consequently, algae do not use the light energy absorbed to grow but only to maintain themselves (Figure 2.1b, dotted line). At a PFD above the compensation point photosynthetic rate increases linearly with PFD and the maximal biomass yield is reached (Figure 2.1b, dotted line). A further increase in PFD results in a gradual decrease in biomass yield because the specific photosynthetic rate saturates and reaches its maximum whereas the specific light absorption rate keeps on increasing with PFD and surplus of absorbed light energy is dissipated as heat.

To assure sufficient mass transfer, a PBR has to be mixed either by gassing or pumping the algae suspension to and from a (de)gassing unit. The mixing leads to a movement of algae through the light path (left column, Figure 2.2). The intensity of mixing and the PBR geometry determine the nature of light/dark (L/D) cycles algae experience. Light/dark cycling influences the overall productivity because algae respond to each (transient) light regime differently (right column Figure 2.2): At over-saturating PFDs the photon supply rate at the PBR surface exceeds the maximal rate of energy consumption, therefore more than 2/3 of absorbed light energy is dissipated in this example (Figure 2.2). In the next layer of the PBR the PFD is sub-saturating and absorbed light energy is used more efficiently. Finally, in the dark layer of the PBR no light energy is available and only stored carbon compounds can be used to provide metabolic energy. However, the exact response seems to deviate from this simplified description especially in the situation of very fast or very slow L/D cycles. This deviation is possibly related to internal metabolic buffers or activation/deactivation effects in certain metabolic processes.

The influence of mixing-induced flashing light on PBR productivity has been reported in several studies and will be discussed in the next section.

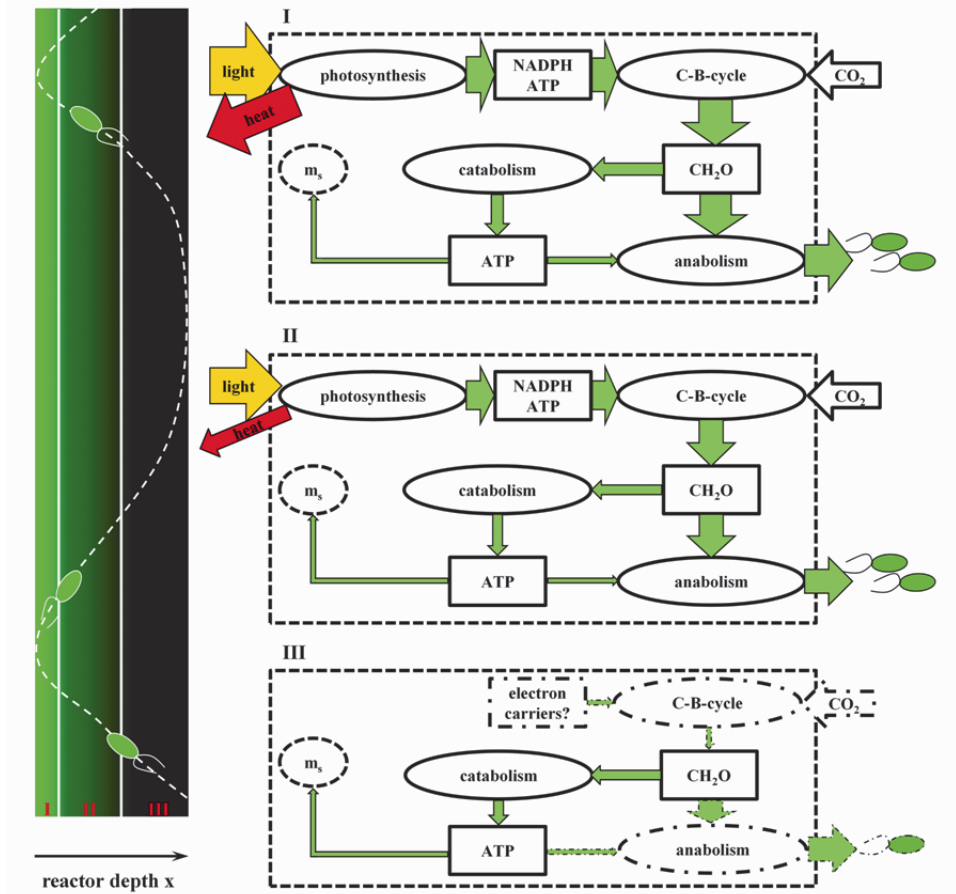


Figure 2.2 Left column: schematic view of a photobioreactor exposed to over-saturating PFD, the dotted line shows a hypothetical trajectory algae may follow due to mixing. I: – zone with over-saturating PFDs (low biomass yield), II zone with saturating and limiting PFDs (high biomass yield), III zone with no light. Right column: illustration of basic metabolic fluxes as they depend on PFD. Abbreviations: m_s – maintenance, C-B-cycle – Calvin-Benson-cycle, CH_2O – basic sugar

Mixing-induced flashing light and photobioreactor productivity

Algae growth experiments in different photobioreactor (PBR) types showed that a change in turbulence, leading to a change in L/D cycling, influenced specific growth rate, photosynthetic efficiency, and PBR productivity.

Hu and Richmond (1996) showed that the increase in gassing rate from 0.6 to $4.2 \text{ L L}^{-1} \text{ min}^{-1}$ resulted in a doubling in volumetric productivity (0.2 to $0.4 \text{ g L}^{-1} \text{ h}^{-1}$). Furthermore, a dark zone was required and the highest yield was achieved under an over-saturating incident photon flux density (PFD). Because of the dark zone, algae moved from an over-saturating PFD near the surface of the PBR to the dark and back,

and, consequently, experienced light/dark (L/D) cycles. Hu and Richmond (1996) suggested that this fast cycling resulted in a flashing-light effect leading to extremely efficient use of light energy by *A. platensis*.

In other experiments with green microalgae an increase in gassing rate (0.33 to 0.66 L L⁻¹ min⁻¹) showed a doubling in volumetric productivity as well (0.05 to 0.1 g L⁻¹ h⁻¹, Meiser et al., 2004). Kliphuis et al. (2010) increased turbulence by change in stirrer speed; a doubling (70 rpm to 140 rpm) resulted in a 5 % increase in biomass yield.

Cycle times between light and dark zones can be decreased by reducing the light path of a PBR, but inconsistent results have been reported in literature: increased biomass yield (Degen et al., 2001; Giannelli et al., 2009; Richmond et al., 2003), constant biomass yield (Zijffers et al., 2010) and decreased biomass yield (Zou et al., 1999).

The effect of turbulence-induced L/D cycles may become visible if algae are exposed to more defined L/D cycles. These L/D cycles can be achieved by introducing static mixers such as baffles. In comparison to random mixing the biomass yield was nearly doubled with introduction of regular mixing (Degen et al., 2001; Laws et al., 1983).

The described PBR studies are difficult to compare because experimental conditions were different: batch versus continuous processes, different microalgae species, different mixing rates, different gas exchange strategies, no consistent reference experiments and different evaluation of PBR performance (volumetric productivity, biomass yield). In these studies an effect of turbulence on biomass yield was observed. Although the existence of a flashing-light effect is suggested, enhanced mass transfer could have contributed to higher biomass yield in more turbulent systems as well.

Not many quantitative descriptions about actual L/D cycles are given in PBR studies. Several new techniques are available to describe mixing in PBRs. With computer automated radioactive particle tracking (CARPT) three types of flow patterns with frequencies of 0.1 Hz, 1 Hz and 10 Hz were identified in a draft-tube and split-column PBR (Luo et al., 2003). A combination of a flow pattern model with an irradiance distribution model (Fernandez, et al., 1997) showed that the higher the biomass concentration in the PBR the greater the changes in PFD experienced by algae.

Flow patterns can also be calculated by computational fluid dynamic (CFD) simulations (Moberg et al., 2011; Perner-Nochta and Posten, 2007; Pruvost et al., 2006, 2008; Wu et al., 2009). These CFD simulations showed the existence of light/dark cycling inside

PBRs. The highest reported frequency was 25 Hz observed in a tubular PBR equipped with a static helical mixer (Perner-Nochta and Posten, 2007).

As an overall conclusion, PBR performance can be optimized based on controlled mixing and L/D cycling. Optimization strategies should be based on reliable mechanistic models that describe the influence of dynamic light regimes on algae growth and algae productivity. And, productivity gain by increased mixing (L/D cycling) should always be balanced with the increase in energy input.

Photosynthesis in dynamic light regimes

In a photobioreactor (PBR) algae will experience changes in photon flux density (PFD) while moving through the PBR. Dynamic light regimes in laboratory experiments are usually simulated in a simplified way by applying square-wave light/dark (L/D) cycles. These experiments are mostly done with dilute cultures to minimize light attenuation; consequently all algae receive a similar controlled light regime. A description of a square-wave L/D cycle is presented in Figure 2.3.

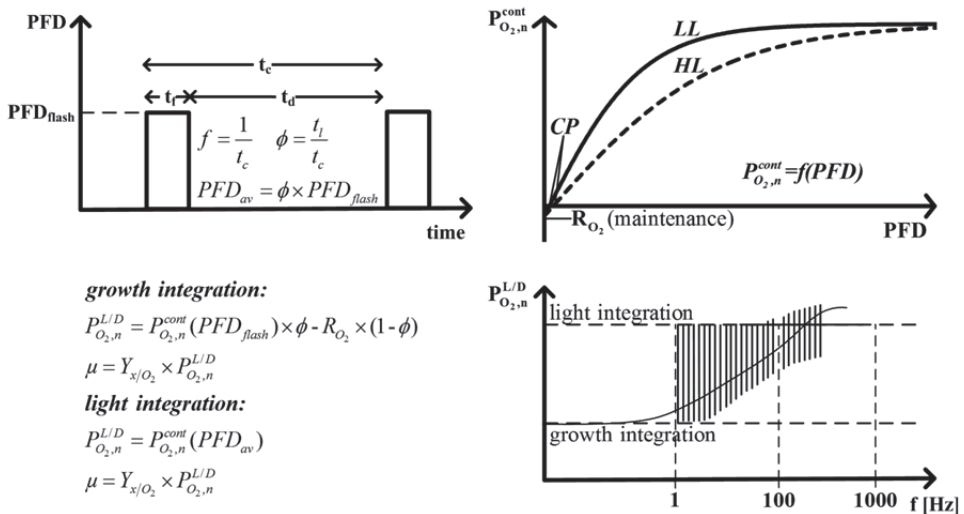


Figure 2.3 Top left: definition of a square-wave light/dark cycle. Top right: net photosynthetic oxygen evolution rate ($P_{O_2,n}^{cont}$) under continuous illumination for high light (HL) and low light (LL) acclimated algae, CP – compensation point. Bottom left: two simple models to describe microalgae growth under flashing light by means of the photosynthetic rate measured under continuous illumination: growth integration versus light integration. Bottom right: schematic representation of growth and light integration in a PBR depending on the flash frequency, grey area shows scatter of results derived from studies about the effect of fluctuating light on algae growth.

The photosynthetic response to a (dynamic) light regime can be measured as oxygen evolution rate or specific growth rate. In these measurements the acclimation state of

algae is important. The acclimation state influences short term oxygen production rate in continuous light, especially at limiting PFDs, as illustrated in Figure 2.3 (top right). Photoacclimation is the change in optical cross section, which dictates light absorption, rate of photosynthesis and biomass yield on light energy. Therefore, photoacclimation to different light regimes will result in different oxygen production rates in the same flashing light regime.

Oxygen evolution rates are measured for short periods of time (1 min – 1 hour) and, consequently, algae cannot acclimate to the light regime tested and the acclimation state (Dubinsky and Stambler, 2009; Falkowski and Raven, 2007) depends solely on the pre-cultivation conditions. In contrast, specific growth rates are measured in (semi)-continuous experiments (repeated batch, chemostat or turbidostat). These continuous experiments last for several days and, as a result, algae will be fully acclimated to the light regime tested.

The effect of intermittent illumination on photosynthetic response must be evaluated by comparison to a reference experiment under continuous illumination. The photosynthetic response to an intermittent light regime can then be described by two simplified and opposite reactions (Figure 2.3, bottom left). First, algae photosynthesis could be assumed to respond to flashing light as it would respond to continuous light of an intensity equal to the (incident) time-averaged PFD. This response to flashing light is also referred to as full light integration (Phillips and Myers, 1954; Terry, 1986) and is beneficial because over-saturating light is diluted in time to sub-saturating light levels. As a consequence of such light dilution algae express a high biomass yield on absorbed light energy, which results, in more efficient growth.

Second, algae photosynthesis could be assumed to respond immediately to the actual PFD received during the flash and is zero or negative during the dark part of the cycle. The photosynthetic rate during the light flash is equal to the corresponding rate under continuous illumination at that PFD. This response to intermittent light will be called growth integration and can be used as a second reference point.

The length of L/D cycles found in PBRs can vary from hundreds of milliseconds to minutes (see section “Mixing-induced flashing light and photobioreactor productivity”). In general, L/D cycles from seconds to minutes ($f < 1$ Hz) lead to growth integration. For flash frequencies greater than 1 Hz the effect is opposite and photosynthetic rate or biomass yield increase in comparison to growth integration. This increase is caused by (partial) light integration, and can be referred to as flashing-light effect. However the

term flashing-light effect is not well defined and we propose that it should only be used to describe full light integration. If full light integration is not reached then rate, or efficiency improvement due to flashing light should be referred to as partial light integration.

Light/dark cycles on a scale of seconds

Lee and Pirt (1981) and Merchuk et al. (1998) showed that a dark period up to 9.2 s could be sustained without reduction of specific growth rate in comparison to continuous illumination. Lee and Pirt (1981) concluded that algae can store sufficient amounts of energy to continue photosynthesis during the dark period. These results, however, could not be confirmed by other studies (Barbosa et al., 2003; Janssen et al. 1999, 2000, 2001). The specific growth rate in intermittent illumination reached values as expected for growth integration. These observations were confirmed for different algae strains (*Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Dunaliella tertiolecta*), which suggests a species independence. However, the biomass yield on light energy was more influenced by L/D cycling because in general it was lower in comparison to the value expected for growth integration (Barbosa et al., 2003; Janssen et al. 1999, 2000, 2001). Therefore, light energy was used less efficiently during light exposure in an intermittent regime than under a constant PFD equal to the flash intensity. This negative effect of L/D cycling was related to an increase in optical cross section (photoacclimation) under intermittent light leading to more photo-saturation during light exposure, which was confirmed by increased non-photochemical quenching and a reduction in quantum yield of photochemistry (Janssen, 2002).

These results suggest that algae are not able to store sufficient energy to use during the dark period. A consequence of the long dark period is that basic carbon compounds, which have been synthesized in the light, will be respired for maintenance purposes and reduce the overall growth rate and biomass yield.

Sub second light/dark cycles

Research about effects of intermittent light on photosynthesis was first mentioned in 1919 (Warburg, 1919). Experiments done during the 19(30)s are reviewed by Myers (1994), but not until the 19(50)s the precision of measurements improved sufficiently to be able to perform adequate research on this effect (Kok and Burlew, 1953).

Experiments in sub-second, intermittent light, both on algae and plants, were re-evaluated by Sager and Giger (1980). Re-evaluation was done to test the basic hypothesis of Rabinowitch (1956) that photosynthetic rate in intermittent light can approach but not exceed the one under continuous illumination with the same time-averaged PFD, which was the case. Furthermore, Sager and Giger (1980) stressed the importance to compare photosynthetic rates from intermittent illumination experiments to rates obtained under time-averaged, continuous PFDs to fully evaluate its effect.

Kok and Burlew (1953) concluded that the light “history” or acclimation state of algae is important for the photosynthetic response to any given light regime. Additionally, results of Grobbelaar et al. (1996) showed a difference in photosynthetic response in continuous light and flashing light for differently acclimated algae (Figure 2.3: top right). Nevertheless, not all studies on sub-second L/D cycles paid attention to the pre-acclimation state of algae used (Table 2.1).

Many L/D cycle experiments have been done as oxygen evolution experiments (Table 2.1). As a consequence algae were not fully acclimated to the light regime applied but to the pre-cultivation conditions, and the exact acclimation state is not known. Vejrazka et al. (2011) showed that under flashing light with a saturating flash but low time-averaged PFD ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) algae acclimate in a PBR to a low PFD. Nevertheless, several studies on L/D cycles were done with high light ($> 500 \mu\text{mol m}^{-2} \text{s}^{-1}$) acclimated algae (Table 2.1). This difference in acclimation state might have influenced results of photosynthetic rate measurements under intermittent light.

Table 2.1: Photosynthetic response and pre-adaptation conditions for different L/D cycle experiments

study		photosynthetic response	pre-adaptation
Kok and Burlew	1953	P_{O_2}	turbidostat → HL
Kok	1956	P_{O_2}	turbidostat → HL
Phillips and Myers	1954	μ	turbidostat
Terry	1986	P_{O_2}	batch culture → LL
Matthijs et al.	1995	μ	turbidostat cultivation
Nedbal et al.	1996	P_{O_2}, μ^1	turbidostat → HL
Grobbelaar et al.	1996	P_{O_2}	(HL and LL) ²
Rosello-Sastre	2010	μ	turbidostat cultivation
Xue et al.	2011	$\mu, Y_{x/ph}$	batch cultivation
Brindley et al.	2011	P_{O_2}	chemostat → LL ³
Vejrazka et al.	2011	$\mu, Y_{x/ph}$	turbidostat cultivation

¹short term growth rate experiments (maximal 4 hours), ²semi-continuous cultivation, dilution of the culture once a day, ³with 12h/12h day/night cycle. Parameters: μ - biomass specific growth rate, P_{O_2} - biomass specific oxygen production rate, $Y_{x/ph}$ - biomass yield on photons.

Full light integration can be achieved in terms of photosynthetic rate or in terms of biomass yield on light energy. Biomass yield is more accurate because it includes absorbed light, biomass concentration and photosynthetic rate (Vejrazka et al., 2011). As a result the biomass yield corrects for a possible change in absorption cross section caused by photoacclimation.

An overview of L/D cycle studies is shown in Table 2.2, which is incomplete for the study of Kok and Burlew (1953) because not all relevant parameters were given for each experiment. Terry (1986) reported pooled data sets only, which do not allow a complete evaluation of the amount of light integration observed. Table 2.2 shows that with decrease from high to low flash frequencies the degree of light integration is decreasing. The flash frequency at which full light integration was observed varied: 1 Hz (Brindley et al., 2011), 5 Hz (Terry, 1986), 14 Hz – 100 Hz (Kok, 1956; Phillips and Myers, 1954; Rosello-Sastre, 2010; Vejrazka et al., 2011), 1 kHz and more (Matthijs et al., 1996; Nedbal et al., 1996). Additionally, Brindley et al. (2011) showed evidence that light integration is enhanced at frequencies above 10 Hz if light was completely absorbed during the measurement.

In order to evaluate light integration the flash time is important as well and, in general it should not be longer than 50 ms (Table 2.2). But the flash time of 50 ms could not be confirmed by Xue et al. (2011) because full light integration was not observed at even

the shortest flash time of 2.5 ms ($\Phi = 0.05$, $f = 20$ Hz). In addition, Nedbal et al. (1996) and Grobbelaar et al. (1996) did not observe full light integration for flash times greater than 1 ms.

Table 2.2 Overview of experimental conditions of L/D cycles and conditions where full light integration was observed.

study		conditions tested		full light integration		
author(s)	year	f^1 [Hz]	PFD_0^2 [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	f [Hz]	t_f [ms]	Φ [-]
Kok and Burlew	1953	max 80	1260	40	?	?
³ Kok	1956	17, 526	1260	16.6	0.47	0.008
Phillips and Myers	1954	1.5 - 144	1150	24 - 144	1	0.025 - 0.1
Terry	1986	0.25 - 7.5	250, 500, 1000, 1750	5	50	0.25
Matthijs et al.	1995	2500 - 25000	460 - 1040	2500 - 25000	0.005	0.0125 - 0.125
Nedbal et al.	1996	0.1 - 10000	130 - 3000	1000	0.5	0.5
Grobbelaar et al.	1996	0.05 - 1000	800	1000	0.5	0.5
Rosello-Sastre	2010	0.17 - 100	1940 (540) ⁵	14 (25)	10 (20)	0.14 (0.5)
Xue et al.	2011	0.01 - 20	150, 390, 1240, 3700	-	-	-
⁴ Brindley et al.	2011	0.1 - 50	680, 1260, 2021	1	50	0.05
Vejrazka et al.	2011	5 - 100	1000	100	1	0.1

The light intensities of the studies before 1986 were recalculated based on the assumption that $1 \text{ W m}^{-2} = 4.8 \mu\text{mol m}^{-2} \text{ s}^{-1}$ frequency range tested, ²incident light intensities tested, ³full light integration at this frequency was only observed at very low average light intensities, ⁴light integration at this frequency was not observed for longer duty cycles, ⁵1940 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ was tested for only one L/D cycle setting, values in brackets indicate results from lower PFD_0 .

Nedbal et al. (1996) and Terry (1986) concluded that duty cycle and incident PFD have little influence on the amount of light integration, but Grobbelaar et al. (1996) and Brindley et al. (2011) showed a duty cycle dependency for different acclimated algae. Terry (1986) concluded that a duty cycle of 0.1 would lead to an optimal biomass yield. A consequence of these observations is that the optimum duty cycle could depend on both flash length and flash intensity but this relation is not well studied nor mathematically described.

Except for the study of Rosello-Sastre (2010) and Brindley et al. (2011) photosynthetic rates under flashing light did not exceed the maximal rates under continuous illumination in accordance with the hypothesis of Rabinowitch (1956). In addition, in Rosello-Sastre (2010) specific growth rates in flashing light even exceeded the maximal growth rate measured under continuous illumination.

The growth rate experiments of Nedbal et al. (1996) showed higher growth rates in intermittent illumination ($f \approx 80$ Hz, $\Phi = 0.5$) in comparison to continuous illumination

with the same time-averaged PFD. However, these specific growth rates were determined in short experiments (≈ 4 hours) and algae were not acclimated to the tested light regime.

As a conclusion the amount of light integration is dependent on the duty cycle, which determines the time-average PFD, but the flash time has to be well balanced with the dark time to allow an optimal usage of absorbed light energy. A long flash time in combination with a short dark time might over-saturate the photosystem and lead to inefficiencies, whereas dark times beyond a certain threshold can lead to inefficiencies because a larger fraction of photosynthetically produced energy carriers will be respired for maintenance. These observations strengthen the hypothesis of Vejrazka et al. (2011) that a (limited) pool of energy carriers is available to allow the dark reaction of photosynthesis to continue for a short time during the dark part of an L/D cycle.

There is no fixed parameter configuration for full light integration: If only results from long-term specific growth rate measurements are considered then a minimum flash frequency of 14 Hz in combination with a flash time of 10 ms was reported to achieve full light integration (Phillips and Myers, 1954; Rosello-Sastre, 2010). If results from short-term oxygen evolution experiments are considered then a minimum flash frequency of 1 Hz with a flash time of 50 ms was reported to achieve full light integration (Brindley, et al., 2010). This variable behavior is illustrated in Figure 2.3 (bottom right) where a range of frequencies could result in a certain degree of light integration but flash frequencies below 1 Hz will lead to growth integration only.

PBR operation by application of flashing light

Progress in LED development through the last decade resulted in efficient, high power LEDs that can be flashed up to a megahertz range with a brightness far exceeding the capacity of the photosynthetic apparatus of green microalgae. These LEDs can be used to provide a photobioreactor (PBR) with artificial illumination based on electricity possibly produced from renewable energy sources.

Sub-second light/dark (L/D) cycles have a positive influence on photosynthetic rate and photosynthetic efficiency as discussed in the previous section. Therefore, it is thinkable to provide flashing light to the PBR if working with artificial illumination. Then the question arises whether flashing artificial light leads to higher photosynthetic rates and PBR productivity than continuous artificial illumination with a photon flux density (PFD) equal to the time-averaged PFD of the flashing light. For this analysis we do not

consider the possible effects related to mixing-induced L/D cycles as discussed in Section 3. In case of (flashing) artificial light the biomass density has to be adjusted as such that the PFD in each layer of the PBR is sufficient to ensure net photosynthesis. The PFD, or time-averaged PFD (PFD_{av}) in case of flashing light, at the backside of the PBR should be equal to the compensation point (PFD_c) which will lead to maximal photobioreactor productivity (Takache et al. 2010). As a result of these PFD requirements, the optimal biomass density will be identical for continuous and flashing light.

The maximal improvement in specific photosynthetic rate by application of flashing light only, is limited, which is illustrated in Figure 2.4. In continuous illumination photosynthetic rate is increasing linear with increase in PFD for low PFDs (Figure 2.1b, Figure 2.4a). Further increase in PFD results in a non-linear increase in photosynthetic rate until a biological maximum is reached. In the best case scenario for flashing light, the photosynthetic rate will increase linearly with increase in PFD until the same biological maximum is reached. This linear increase until the biological maximum is only possible if the light flashes are perfectly timed with the redox state of the reaction centers. In other words, single-turnover flashes must hit the photosystem in the exact moment when it re-opens and already excited photosystems are not hit by the light flash.

The spatially averaged photosynthetic rate for continuous illumination and flashing light can be calculated with the assumption that mixing induced L/D cycles do not have an influence (Figure 2.4b). The resulting spatial photosynthetic rate can be integrated over the PBR depth and is 10 % higher for flashing light in comparison to continuous light in this scenario. The photosynthetic rate in this scenario was calculated based on the hyperbolic tangent model proposed by Jassby and Platt (1976). The maximal oxygen production rate in this model is species dependent and the initial slope varies depending on the acclimation state of the algae (Figure 2.3, top right), therefore the 10 % increase shows an order of magnitude in increase which can be expected.

The optimal biomass density is equal under continuous and flashing light; therefore the volumetric productivity of the PBR will be 10 % higher under flashing light in this scenario. The conclusion is that the application of flashing light alone to a PBR has a limited effect on photosynthetic rate and productivity.

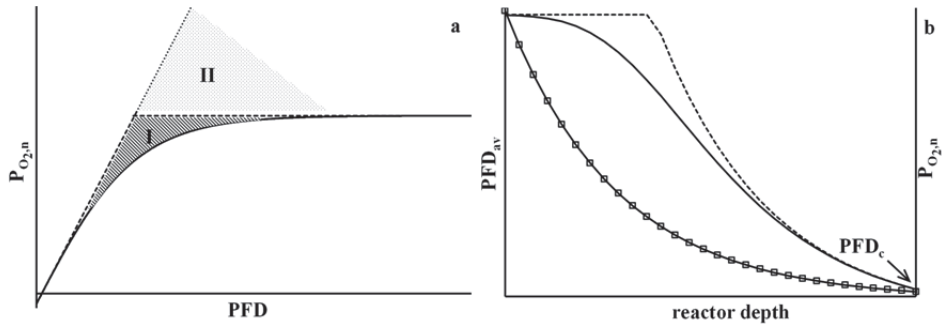


Figure 2.4 a) specific photosynthetic oxygen evolution rate versus incident photon flux density, — normal response for a low light acclimated algae, --- ideal response in flashing light until biological, maximal rate is reached, ... theoretic extension of ideal response if biological maximum could be increased, I area for possible photosynthetic rate increase by application of flashing light only, II in this area rate cannot be increased due to biological constraints. b) time-averaged photon flux density (\square , see Figure 2.1, 0.5 g/L biomass) versus PBR depth and photosynthetic rate (i.e. oxygen evolution rate) versus photobioreactor depth for — normal response and --- ideal response, PFD_c : photon flux density at compensation point.

General discussion

Several studies showed that increased turbulence in a photobioreactor (PBR) has an influence on algae growth and biomass yield on light energy. However, it is difficult to determine the extent of improvement that can be achieved due to light integration or improved mass transfer. Low mixing rates will result in low mass transfer and can lead to oxygen inhibition or carbon dioxide limitation. An enhanced mixing in this situation will improve the biomass yield on light energy. As a conclusion, sufficient information about mass transfer (dissolved oxygen and carbon dioxide concentration) should be available in PBR studies to be able to exclude their influence on productivity.

One of the hypotheses for the positive effect of flashing light with over-saturating PFDs is reduced photoinhibition in comparison to continuous light (Nedbal et al., 1996). Also this hypothesis and its dependency on the flash intensity have to be studied in more detail.

The incident time-averaged PFD, which is set by the flash PFD and duty cycle, is of importance to achieve (full) light integration. If the incident, time-averaged PFD is non-saturating then full light integration is achieved at flash times equal or smaller than 50 ms (Brindley et al. 2011; Phillips and Myers, 1954; Vejrazka et al., 2011). In addition, Xue et al. (2011) showed that with a decrease in duty cycle (flash frequency and flash PFD constant) light integration increased. The implications on a PBR level are that higher biomass concentrations (larger dark zone) are allowed for an efficient

operation provided mixing is fast enough to generate such sub-second L/D cycles (Dubinsky and Stambler, 2009).

The concept of light integration suggests that algae can store energy during the light part of the L/D cycle and use it during the dark part. As a consequence of a dark zone the PBR can be operated with higher biomass concentrations, and the overall productivity of the system will be enhanced. Nevertheless, it will be difficult to achieve full light integration in a PBR because of the fast mixing required. To achieve full light integration in a PBR a minimal frequency of 14 Hz is needed (Table 2.2). An intermediate stage between growth integration and full light integration may provide a viable scenario for short light path PBRs. But, biomass yield and productivity can only be optimized if this intermediate stage can be quantified. This quantification is important because with increase in biomass concentration (increase in dark zone) the relative amount of light energy needed for maintenance will increase as well. And, at a threshold biomass concentration eventually negate the positive effect of enhanced biomass yield due to L/D cycling.

To fully exploit the beneficial effect of mixing induced L/D cycles additional research is necessary in two major fields: improvement of hydrodynamic models to characterize mixing induced L/D cycles in any given PBR system and characterization of algae response to flashing light. This characterization has to include the interdependency between duty cycle, flash intensity, flash time and dark time to achieve an optimal biomass yield. In addition, research should move towards L/D cycle studies under full light attenuation because this is the relevant light regime for PBR operation practice.

Mathematical models that rely on empirical parameters (Zonneveld, 1998) or mechanistic models (Eilers and Peeters, 1988; Rubio et al., 2003) that can describe growth and productivity of microalgae under dynamic light regimes can be improved and further developed based on data from L/D cycle experiments. These models will finally help to describe to what extent L/D cycles influence PBR productivity and new PBR design guidelines and optimal operation conditions can be developed.

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Chapter 3

Photosynthetic efficiency of *Chlamydomonas reinhardtii* in flashing light

Abstract

Efficient light to biomass conversion in photobioreactors is crucial for economically feasible microalgae production processes. It has been suggested that photosynthesis is enhanced in short light path photobioreactors by mixing-induced flashing light regimes. In this study, photosynthetic efficiency and growth of the green microalga *Chlamydomonas reinhardtii* were measured using LED light to simulate light/dark cycles ranging from 5 to 100 Hz at a light/dark ratio of 0.1 and a flash intensity of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Light flashing at 100 Hz yielded the same photosynthetic efficiency and specific growth rate as cultivation under continuous illumination with the same time-averaged photon flux density (i.e. $100 \mu\text{mol m}^{-2} \text{s}^{-1}$). The efficiency and growth rate decreased with decreasing flash frequency. Even at 5 Hz flashing, the rate of linear electron transport during the flash was still 2.5 times higher than during maximal growth under continuous light, suggesting storage of reducing equivalents during the flash, which are available during the dark period. In this way the dark reaction of photosynthesis can continue during the dark time of a light/dark cycle. Understanding photosynthetic growth in dynamic light regimes is crucial for model development to predict micro algal photobioreactor productivities.

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Introduction

The production of bulk chemicals and biofuels from renewable feed stocks can only compete with fossil feed stocks if a low production cost at large-scale can be achieved. Low production costs of algal feed stocks depend, among others, on high photobioreactor (PBR) productivities, thus efficient conversion of light energy into biomass and product (Norsker et al., 2010). For the optimal design of microalgae photobioreactors a high photosynthetic efficiency (biomass yield) is necessary to achieve high areal productivities.

One of the least understood phenomena is the influence of fluctuating light regimes on growth and productivity of microalgae. A photobioreactor (PBR) exposed to light has an inherent light gradient due to light attenuation by the algae. This light gradient reduces the photic zone where net photosynthesis and growth occurs. Due to mixing the individual algae cells alternate between the photic zone and the dark zone, thus experiencing light/dark (L/D) cycles. A big challenge is to understand the impact of these L/D cycles on biomass growth since the design and operation of a PBR depends on the availability of good mechanistic models predicting the photosynthetic response under any given dynamic light regime.

Dynamic light regimes in photobioreactors can be simplified by square-wave L/D cycles. Such an intermittent light regime can then be defined by the flash time (t_l , i.e. length of the flash), the dark time (t_d), the cycle time ($t_c = t_l + t_d$), the duty cycle ($\phi = t_l/t_c$) and flash frequency ($f = 1/t_c$).

High photon flux densities (i.e. over-saturating illumination) saturate the photosystem so that the biomass yield on light energy decreases with increasing photon flux density (PFD). It has been shown that under sub-second L/D cycles microalgae growth rates, and possibly photosynthetic yields, can be improved under over-saturating illumination. At sufficiently fast cycles, and optimized L/D ratios, full light integration has been observed (Grobelaar et al., 1996; Kok and Burlew, 1953; Kok, 1956; Matthijs et al., 1996; Nebal et al., 1996; Phillips and Myers, 1954; Terry, 1986). Light integration is the perception of fluctuating light as continuous light of an intensity equal to the time-averaged PFD for one L/D cycle (Terry, 1986). This time-averaged PFD is no longer over-saturating, so that the biomass yield on light energy increases.

The flash frequencies (i.e. number of flashes per second) reported to reach full light integration range from 5 Hz (Terry, 1986), 24 - 100 Hz (Kok and Burlew, 1953; Phillips

and Myers, 1954) to 1 kHz and more (Matthijs et al., 1996; Nebal et al., 1996), in other words the threshold for full light integration is not well determined.

So far, no reliable data have been reported on the photosynthetic efficiency of algae growth under flashing light since most of the studies have been done based on short term photosynthetic oxygen evolution measurements in which the algae did not have time to acclimate to the light regime imposed (Brindley et al., 2011; Grobbelaar et al., 1996; Kok and Burlew, 1953; Kok, 1956; Nebal et al., 1996; Terry, 1986) or in batch experiments with continuous changes in light regime (Xue et al., 2011). Photoacclimation will result in a change of the specific light absorption coefficient (optical cross section) and determine the photosynthetic efficiency. Longer term experiments only report about photosynthetic rates (Matthijs et al., 1996; Phillips and Myers, 1954) but not on photosynthetic efficiency or biomass yield on light energy for which the light absorption capacity should also be known.

Nevertheless, studies in short light path (SLP) panel photobioreactors showed, that photosynthetic efficiency and productivity are improving with increase in turbulence or L/D cycling (Meiser et al., 2004; Hu and Richmond, 1996), whereas the increase was greatest under incident PFDs that would be over-saturating if used as continuous illumination. Recent work in SLP photobioreactors with green microalgae and over-saturating incident PFDs showed that an increase in gassing rate (Zijffers, 2009) or stirrer speed (Kliphuis et al., 2010a) or a decrease of light path (Zijffers et al., 2010) did result in a minor improvement in photosynthetic efficiency at best. Contrary, Gordon and Polle (2007) argued that the yield of current photobioreactor systems could be increased up to 50 fold by applying micro-second L/D cycles with LEDs. In this study we present a set of long-term turbidostat experiments performed under well-defined, sub-second L/D cycles in combination with reference experiments under continuous illumination. The presented experiments were intended to gain further insight into the effect of L/D cycling on algal growth and productivity. Based on the measured growth rates and light absorption rates we calculated the biomass yield on light energy and the amount of light integration for different flash frequencies tested was assessed.

Material and Methods

Organism and medium

Chlamydomonas reinhardtii CC-124 wild type mt- 137c was kindly provided by Thomas Happe from Bochum University. The microalgae were cultivated in a Sueka high salt

(HS) based liquid medium, composed of (amounts in g L^{-1}): NH_4Cl , 1.450; KH_2PO_4 , 0.720; K_2HPO_4 , 1.440; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.280; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.057; $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 0.067 and 10 mL L^{-1} of a 100 times concentrated Hutner's trace elements solution (Hutner et al., 1950). Axenic cultures were maintained in shake flasks placed on an orbital shake incubator at pH 7, 25 °C, air enriched with 2.5 %_{v/v} CO_2 , and 60–80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ continuous illumination for at least one week before use as photobioreactor (PBR) inoculum. The PBR medium contained additionally 0.420 g L^{-1} of NaHCO_3 .

Photobioreactor set-up

The PBR system (Photobioreactor FMT 150, Nedbal et al. (2008)) was purchased from Photon System Instruments (PSI) and equipped with a blue-red LED panel emitting at 450 nm and 630 nm. The PBR was operated as a turbidostat (axenic); each experiment was done in a biological duplicate in two independent PBRs and started with a dry weight concentration of $\approx 0.025 \text{ g}_{\text{bm}} \text{ L}^{-1}$. To enable the culture to acclimate to each light regime, the light transmission during each turbidostat experiment was kept at 60 %, thus minimizing mutual shading while still having a sufficient biomass density allowing accurate analysis of PBR productivity.

Temperature was regulated at 25 °C and the culture was mixed by sparging air at a flow rate of 1 $\text{L}_{\text{gas}} \text{L}_{\text{culture}}^{-1} \text{ min}^{-1}$. The photobioreactor cuvette (1 x w x h = 100 x 25 x 195 mm) was filled up to 145 mm height resulting in an illuminated liquid working volume of 360 mL. The pH was kept constant at 7.0 via pulse-wise CO_2 addition into the air stream.

The turbidity was controlled by measuring the photon flux density (PFD) at the rear side of the PBR cuvette with a Hamamatsu S1227-33BR photodiode. The amplified signal was processed via an A/D converter (NI USB-6008, National Instruments™, USA) and a LabView (National Instruments™, LabView 7.1) program.

The specific growth rate ($\mu(t)$, $\text{g}_{\text{bm}} \text{ g}_{\text{bm}}^{-1} \text{ h}^{-1}$) was calculated according to the biomass mass balance (equation (3.1)):

$$\frac{dV_R c_x^R}{dt} = \mu \cdot c_x^R \cdot V_R - \dot{F}_{out} \cdot c_x^{out} \quad (3.1)$$

Assuming a constant PBR volume (V_R , L) and steady state, equation (3.1) can be simplified to equation (3.2), where (c_x^R , $\text{g}_{\text{bm}} \text{ L}^{-1}$) and (c_x^{out} , $\text{g}_{\text{bm}} \text{ L}^{-1}$) are the biomass

concentrations in the PBR and overflow at the time of sampling (daily) and ($\dot{F}_{out}(t), Lh^{-1}$) is the outgoing flow rate.

$$\mu(t) = \frac{\dot{F}_{out}(t)}{V_R} \cdot \frac{c_x^{out}}{c_x^R} \quad (3.2)$$

An average for six hour intervals was calculated. The final steady state growth rate was calculated from the six hour intervals for the last 72 h of the cultivation during which the culture was in steady state. To assess if steady state was reached, cell number, average cell size and optical density inside the photobioreactor and overflow had to stay constant for at least 72 h.

Light regime

The tested light regimes are summarized in Table 3.1. Terry (1986) determined a favorable duty cycle (Φ) of 0.1, which was chosen for all flashing light experiments in this study. The flash frequency was set in the range from 5 Hz to 100 Hz, taking into consideration that in current PBR designs L/D cycles will not exceed ≈ 25 Hz as shown in measurements and CFD simulations (Luo et al., 2003; Perner-Nochta and Posten, 2007).

The PFD in the experiment reference 2 is saturating and was chosen to determine the maximum specific growth rate ($\mu(t), g_{bm} g_{bm}^{-1} h^{-1}$) without the influence of light inhibition that does occur at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (results not shown; Janssen et al. (2000)).

Table 3.1: Light regimes tested, each experiment was done in a biological duplicate in two independent photobioreactors.

Experiment*	PFD₀	ϕ	f	t_l
	<i>[$\mu\text{mol m}^{-2} \text{s}^{-1}$]</i>	<i>[-]</i>	<i>[Hz]</i>	<i>[ms]</i>
Reference 1	100	1.0	0	-
Reference 2	500	1.0	0	-
Reference 3	1000	1.0	0	-
5Hz	1000	0.1	5	20
10Hz	1000	0.1	10	10
50Hz	1000	0.1	50	2
100Hz	1000	0.1	100	1

*reference 3 was estimated based on reference 2 (see results and discussion for more details), PFD₀ – incident (flash) photon flux density from 400 to 700 nm, Φ – duty cycle, f – flash frequency, t_l – flash length

Light measurement

Under continuous illumination the incident photon flux density (PFD_0 , $\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured at the position of the light exposed surface of the photobioreactor cuvette at 35 equally distributed points and averaged into PFD_0 . This was done with a LiCor 190-SA 2π PAR (400-700 nm) quantum sensor (LiCor, USA) before and after each experiment. The relative distribution of the photon flux density across the illuminated surface can be found in the appendix.

The spectral distribution (400-700 nm) of the light source was measured at a reference position with a fiber optic CCD based spectroradiometer (AvaSpec-2048 detector, Fiber FC-IR100-1-ME, Avantes, The Netherlands). The reference position was an arbitrarily chosen but fixed position at the rear side of the PBR cuvette. The spectral distribution was measured without the PBR cuvette in intervals ($\Delta\lambda$) of 1 nm as the spectral photon flux density ($PFD_{ref}(\lambda)$, $\mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$). The spectral measurement was normalized for the PAR range to obtain the normalized emission spectrum ($E_{n,PAR}(\lambda)$, nm^{-1}) using equation (3.3).

$$E_{n,PAR}(\lambda) = \frac{PFD_{ref}(\lambda)}{\sum_{\lambda=400}^{700} PFD_{ref}(\lambda) \cdot \Delta\lambda} \quad (3.3)$$

The absolute spectrum of the incident PFD on the PBR surface ($PFD_0(\lambda)$) was calculated by multiplying the incident PFD (PFD_0) with the normalized emission spectrum ($E_{n,PAR}(\lambda)$).

The PAR quantum sensor measurements at the PBR surface and the Avantes spectroradiometer (SRM) measurements at the reference position determined under continuous light were correlated with each other (cf , equation (3.4)).

$$cf = \frac{\sum_{\lambda=400}^{700} PFD_{ref}(\lambda) \cdot \Delta\lambda}{PFD_0} \quad (3.4)$$

In this way, the SRM measurements could be used to determine the time-averaged PFD at the PBR surface under flashing light ($PFD_{0,flash}$, $PFD_{0,flash}(\lambda)$). The time-averaged PFD ($PFD_{0,flash}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$, equation (3.5)) at the PBR surface was calculated based on the reference spectrum for flashing light ($PFD_{ref,flash}(\lambda)$, $\mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$).

$$PFD_{0,flash} = \frac{\sum_{\lambda=400}^{700} PFD_{ref,flash}(\lambda) \cdot \Delta\lambda}{cf} \quad (3.5)$$

The normalized emission spectrum for flashing light ($E_{n,PAR,flash}(\lambda)$, nm^{-1}) was also calculated according to equation (3.3) but with the values for flashing light. Finally, the absolute spectrum of the time-averaged PFD on the PBR surface ($PFD_{0,flash}(\lambda)$, $\mu mol m^{-2} s^{-1} nm^{-1}$) was calculated by multiplying the total time averaged photon flux density ($PFD_{0,flash}$) with the normalized emission spectrum under flashing light ($E_{n,PAR,flash}(\lambda)$).

The light transmission during the cultivations was verified with the SRM measurements: prior to inoculation a blank spectral photon flux density ($PFD_{ref,PBR}^0(\lambda)$) was measured (with PBR cuvette, growth medium and mixed by aeration). The quotient of the blank measurement and the measurement during the cultivation ($PFD_{ref,PBR}^1(\lambda)$) allowed for the calculation of light transmission at each wavelength interval.

Biomass determination

For dry weight measurement Whatman GF/F glass microfiber filters (\varnothing 55 mm, pore size = 0.7 μm) were dried (24 h, 95 °C), cooled down to room temperature in a desiccator (> 2 h) and weighed. The sample (< 5 mg dry weight) was filtrated with a constant vacuum (0.44 bar, absolute) and washed three times with MilliQ water, dried (24 hours, 95 °C), cooled down in a desiccator (> 2 h) and weighted again. The weight difference between empty and algae containing filter was the dry weight. Each sample was measured in triplicate.

Cell size and cell numbers were measured with a Beckman Coulter Multisizer 3 (Beckman Coulter, USA, 50 μm aperture tube). Based on cell number and cell size a total biovolume (mL cells mL⁻¹ of suspension) was calculated.

Dry weight specific absorption coefficient

Light absorption by algae was measured in a specialized spectrophotometer set-up to minimize the effect of light scattering on the absorption measurement. A sample (\approx 15 mL) was centrifuged (15 min, 4500 rpm, 4 °C) and the pellet was re-suspended in the supernatant to reach a final extinction at 680 nm (chlorophyll absorption peak) between 1.8 and 2.2, as measured in a 1 cm cuvette in a spectrophotometer (Beckman DU[®]640, Beckman Coulter, USA). The absorbance was measured with a fiber optic CCD based spectrophotometer (Avantes, The Netherlands). The sample containing cuvette (Hellma, 100.099-OS, 2 mm light path) was illuminated with an AvaLight-Hal light source via a FC-IR600-1-M fiber equipped with a collimating lens. An integrating sphere (AvaSphere-50) was directly placed behind the cuvette and connected to an

Avantes Avaspec-2048 detector via a FC-IR600-1-M fiber. The resulting absorbance was measured from 400 nm to 750 nm ($\Delta\lambda = 1 \text{ nm}$). The average absorbance from 740 nm -750 nm was subtracted from the absorbance between 400 nm and 700 nm, thus correcting for residual scattering (Dubinsky et al., 1986). The wavelength dependent dry weight specific absorption coefficient ($a_{dw}^*(\lambda)$, $m^2 g_{bm}^{-1}$, equation (3.6)) was calculated based on the absorbance (ABS_λ) at wavelength λ , the dry weight (c_x , $g_{bm} m^{-3}$), the light path of the cuvette (l , m), and 2.3026 (conversion factor common logarithm to the natural logarithm):

$$a_{dw}^*(\lambda) = \frac{ABS_\lambda \cdot 2.3026}{c_x \cdot l} \quad (3.6)$$

From $a_{dw}^*(\lambda)$ the average dry weight specific absorption coefficient (\bar{a}_{dw}^*) was calculated (equation (3.7)) with n the number of intervals in the measured range.

$$\bar{a}_{dw}^* = \frac{\sum_{\lambda=400nm}^{700nm} a_{dw}^*(\lambda)}{n} \quad (3.7)$$

Biomass yield on light energy

The light absorption rate inside the photobioreactor ($r_{ph,tot}$, $mol s^{-1}$, equation(3.8)) was calculated based on the measured light spectra (see light measurement), the illuminated surface (A_{light} , m^2) and conversion to mol (1×10^{-6}):

$$r_{ph,tot} = A_{light} \cdot 1 \times 10^{-6} \cdot \sum_{\lambda=400nm}^{700nm} i \cdot \Delta\lambda \cdot \left(1 - \frac{PFDF_{ref,PBR}^1(\lambda)}{PFDF_{ref,PBR}^0(\lambda)} \right) i = PFDF_0(\lambda), PFDF_{0,flash}(\lambda) \quad (3.8)$$

The biomass yield on light energy ($Y_{x/ph}$, $g_{bm} mol^{-1}$, equation (3.10)) is the quotient of the PBR biomass production rate (r_x , $g_{bm} s^{-1}$, equation (3.9)) and the photon absorption rate ($r_{ph,tot}$, $mol s^{-1}$).

$$r_x = \mu \cdot c_x^R \cdot V_R \quad (3.9)$$

$$Y_{x/ph} = r_x / r_{ph,tot} \quad (3.10)$$

Results and discussion

In previous studies on the flashing light effect, the biomass yield on light energy has only been reported once (Xue et al., 2010), unfortunately under batch conditions with rapidly changing light regime. It is an important measure to evaluate photosynthetic growth under different light regimes because it can be extrapolated to outdoor photobioreactor (PBR) productivity. Here we focus specifically on the biomass yield on light energy for a set of turbidostat experiments under flashing light and compare it to reference turbidostat experiments under continuous illumination.

Biomass yield on light energy

The biomass yield on light energy under continuous illumination was $0.49 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ for $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and $0.86 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ for $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Figure 3.1). The biomass yield for $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ continuous illumination, equivalent to the flash PFD, was calculated based on the yield measured at $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ resulting in $0.24 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$. It was assumed that when increasing the PFD from $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ to $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ all additional light energy will be dissipated as heat and fluorescence. In this way it was possible to determine a hypothetical maximal biomass yield for continuous illumination at $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ without the influence of light inhibition. Due to light inhibition the “real” yield will be lower.

The biomass yield on light energy for the same alga recalculated from experiments under continuous illumination with white LEDs by Takache et al. (2010) was $1.11 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ for $110 \mu\text{mol m}^{-2} \text{ s}^{-1}$, which is higher than in our study. The difference could be caused by the light gradient in the experiments of Takache et al. (2010). In their experiments the photon flux density (PFD) leaving the PBR was $10 \mu\text{mol m}^{-2} \text{ s}^{-1}$, thus the average PFD was much lower than in our study which could have resulted in the higher yield. Another explanation could be related to a larger fraction of PAR photons in the blue range (400 nm – 500 nm) in the current study (62 % of total photons) in comparison to the fraction of blue light of the white LEDs (32 % of total photons) used by Takache et al. (2010). Blue light is used at a lower efficiency than orange/red light above 550 nm (Emerson and Lewis, 1943; McCree, 1972).

Under flashing light the biomass yield on light energy increased with increasing flash frequency, whereas it was always greater than the estimated yield ($0.24 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$) for reference 3, continuous illumination at $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Figure 3.1). In other words, the same amount of photons supplied on time average resulted in different yields. To

evaluate the extent of light integration the results have to be compared to the results under $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ continuous illumination (reference 1). At 5 Hz the yield was about 50 % of the yield obtained under $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ continuous illumination. At a frequency of 100 Hz the yields were equal and full light integration was reached.

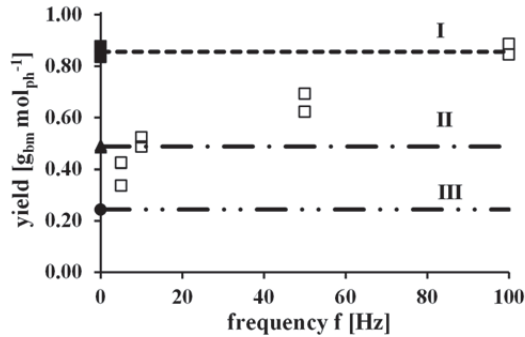


Figure 3.1 Biomass yield on light energy for reference cultivation and flashing light regime, ■ (I) $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ continuous illumination (reference 1), ▲ (II) $500 \mu\text{mol m}^{-2}\text{s}^{-1}$ continuous illumination (reference 2), ● (III) estimation for $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$ continuous illumination (reference 3), □ flashing light, for experimental conditions see Table 3.1

These results show that the flash time has to be maximally 1 ms to reach full light integration in terms of biomass yield, which is in agreement with other studies (Kok and Burlew, 1953; Phillips and Myers, 1954) where full light integration was achieved at the same flash length but in terms of specific growth rate instead of yield. However, the frequency to achieve full light integration may vary and might even be lower than 100 Hz if the flash time is sufficiently short (Phillips and Myers, 1954). Faster flash frequencies with flash times smaller than 1 ms will lead to full light integration as already demonstrated by Matthijs et al. (1996) and Nedbal et al. (1996). In these studies the photosynthetic rates in intermittent light never exceeded the rates in continuous light with the time-averaged illumination.

In Xue et al. (2010) full light integration was not achieved, neither in terms of growth rate nor in terms of biomass yield for the frequency range tested (0.01 -20 Hz). However, they showed that the greatest improvement of biomass yield was achieved under the highest frequency (20 Hz), which is in agreement with the results presented here.

Specific growth rate, dry weight and cell volume

The specific growth rate, dry weight and cell volume were determined for the reference experiments with continuous illumination as well as for the different flash frequencies (Table 3.2). The maximum growth rate measured under $500 \mu\text{mol m}^{-2}\text{s}^{-1}$ (reference 2)

was 0.131 h^{-1} which corresponds well with the previously described maximum growth rate of *Chlamydomonas reinhardtii* (Janssen et al. (2000): 0.132 h^{-1}). The specific growth rate under $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ continuous illumination was 0.072 h^{-1} and this is also the growth rate to be expected for full light integration under flashing light, because the time-averaged PFD under flashing light was $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

The specific growth rate under flashing light increased with increase in flash frequency and at 100 Hz full light integration was achieved as already seen in the biomass yield on light energy data. At 10 Hz and 50 Hz the growth rates were similar but biomass concentrations (Table 3.2) were different and full light integration was not reached, yet. The change in frequency in this range did not seem to have a strong influence on the specific growth rate in contrast to its effect on the biomass yield. The biomass yield showed less deviation because variations in biomass concentration were compensated by opposite changes in growth rate. Therefore, the biomass yield is a better parameter to evaluate the extent of light integration, since it is also indicative for biomass concentration.

The light transmission (i.e. turbidity) through the PBR was controlled based on the turbidostat controller. The light transmission is determined by the biomass concentration and the light absorbing properties of the algae. Since the absorptive properties were similar (as will be discussed below) only biomass concentration determined light transmission through the PBR. The light transmission in experiment 11 (100 Hz, Table 3.2) was controlled at 75 % instead of 60 %, thus the biomass concentration was lower in comparison to experiment 12, but also the growth rate was unexpectedly lower. In experiment 12 the specific growth rate was even higher than in the reference 1 cultivation. The data presented in Table 3.2 shows differences between the replicates, which could be related to biological variations and experimental error (including inconsistencies in the functioning of the turbidostat controller). Considering the fact that the biomass yield on light energy showed less variation between the biological replicates, it seems plausible that this constant biomass yield can be reached with different combinations of growth rate and biomass concentration.

Finally, we observed a trend towards an increase in cell size with increase in growth rate. At 5 Hz (lowest growth rate) the cells were smallest whereas under $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ continuous light (highest growth rate) the cells were biggest. At 100 Hz the cells had roughly the same size and same growth rate as under $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ continuous illumination. At this point of the research there is no explanation of the observed trend.

Table 3.2 Specific growth rate, dry weight, cell size, cell number and biomass yield on light energy for reference and flashing light experiments shown for two independent photobioreactor runs, the standard deviation for μ and the dry weight was calculated as described in material and methods.

#	PFD ₀ [$\mu\text{mol m}^{-2} \text{s}^{-1}$]	f [Hz]	μ [h^{-1}]	dw [g L^{-1}]	cellsize [μm^3]	cell number [# cells 10^5 ml^{-1}]	yield [$\text{g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$]
1	100	0	0.073 ± 0.008	0.053 ± 0.001	133 ± 7	9.51 ± 0.75	0.84
2	100	0	0.070 ± 0.006	0.060 ± 0.004	154 ± 6	8.52 ± 0.26	0.88
3	500	0	0.123 ± 0.004	0.094 ± 0.003	211 ± 18	8.76 ± 0.25	0.48
4	500	0	0.139 ± 0.011	0.100 ± 0.000	222 ± 8	9.00 ± 0.44	0.49
5	1000	5	0.041 ± 0.006	0.056 ± 0.000	67 ± 2	14.60 ± 0.95	0.42
6	1000	5	0.039 ± 0.006	0.051 ± 0.002	69 ± 7	13.2 ± 1.28	0.34
7	1000	10	0.061 ± 0.007	0.049 ± 0.001	85 ± 7	10.70 ± 0.86	0.49
8	1000	10	0.057 ± 0.004	0.045 ± 0.001	93 ± 3	9.52 ± 0.27	0.52
9	1000	50	0.062 ± 0.005	0.061 ± 0.000	133 ± 6	8.87 ± 0.80	0.69
10	1000	50	0.061 ± 0.002	0.061 ± 0.002	134 ± 3	9.19 ± 0.25	0.62
11	1000	100	0.074 ± 0.006	0.043 ± 0.002	141 ± 6	6.38 ± 0.25	0.84
12	1000	100	0.089 ± 0.003	0.059 ± 0.001	105 ± 6	10.50 ± 0.23	0.89

The standard deviation for the cell size and cell number is based on the average of 3 samples for the last 72 h of cultivation

Absorption characteristics and pigmentation

The parameters reported in literature for light full light integration in flashing light vary by as much as an order of magnitude. This non-uniform photosynthetic response might result from different light acclimation states of the algae prior to the intermittent light exposure. In the study of Kok and Burlew (1953) it was already mentioned that the light history of the cells is important. In this study we allowed the algae to acclimate to each light regime tested and the absorption characteristics were measured to assess the acclimation state.

The average dry weight specific absorption coefficient under low light continuous illumination ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) was $0.233 \text{ m}^2 \text{ g}_{\text{bm}}^{-1}$, which was nearly twice as high as under high light continuous illumination ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$, $\bar{a}_{\text{dw}}^* = 0.127 \text{ m}^2 \text{ g}_{\text{bm}}^{-1}$). In the flashing light regime the average absorption coefficient was independent from the flash frequency in the range tested (Figure 3.2a) and it was about the same value as for $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous light. The algae cultivated under flashing light were always acclimated to a low PFD (PFD_{av}), or in general to a growth limiting PFD. In Figure 3.2b the wavelength dependent dry weight specific absorption coefficient is shown and normalized to the value measured at 680 nm. In this way it is possible to determine if the ratio between carotenoids and chlorophylls changed. Except for $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous light all the curves overlap, indicating that the algae were acclimated to the same light regime in each experiment (Figure 3.2b). For $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous

light the peak height in the blue range is increased, which is a sign for increased carotenoid production, thus protection against saturating PFDs to which this culture was exposed.

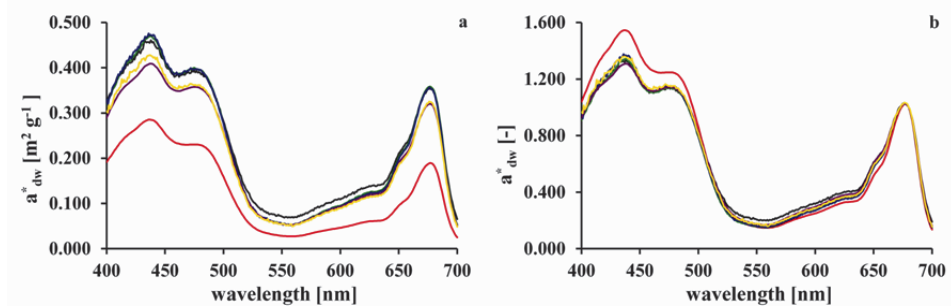


Figure 3.2 (a) wavelength dependent dry weight specific absorption coefficient, (b) wavelength dependent dry weight specific absorption coefficient normalized to dry weight specific absorption coefficient at 680 nm (chlorophyll absorption), each line represents the average for the biological duplicate — reference 1 — reference 2 — 5 Hz — 10 Hz — 50 Hz — 100 Hz

Our findings on the acclimation state in flashing light in combination with the biomass yield on light energy clearly show that the efficiency to use the supplied light was only influenced by the flash frequency and not the capacity to harvest light under low light continuous and intermittent illumination. However, at a saturating, continuous illumination of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ the capacity to harvest light was reduced due to a reduced specific absorptive area.

The acclimation state and, as such, the light absorbing and photosynthetic capacity might have played an important role in previous studies. Since they remain largely unaddressed comparison is difficult. The oxygen evolution experiments of Grobbelaar et al. (1996) and Nedbal et al. (1996) showed full light integration only for frequencies greater than 1000 Hz, whereas Terry (1986) reported full light integration for flash frequencies below 10 Hz for varying over-saturating, incident PFDs and duty cycles. In Nedbal et al. (1996) the algae were acclimated to the time-averaged PFD of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was saturating. In the study of Terry (1986), on the other hand, the algae used for the flashing light experiments were acclimated to a very low PFD whereas time-averaged PFDs in the flashing regime were partly in an over-saturating range, which could have influenced their results.

Estimation of photosynthetic electron transport under flashing light

Photoautotrophic microalgal growth can only be sustained if a sufficient amount of reducing equivalents and energy is produced in the light reaction of photosynthesis. In

flashing light this reaction can only occur during the light flash, whereas the light independent reactions (i.e. carbon fixation and biosynthesis of macromolecules) that lead to growth can also be sustained during the dark if enough reducing power and energy is available throughout the whole dark period. In case of full light integration the production rate of reducing equivalents must be higher during the light flash under flashing light in comparison to growth under continuous illumination at the flash intensity. For this reason we calculated the reducing equivalent production rate based on the stoichiometry for growth on ammonium and the different, measured specific growth rates ($\mu, \text{Cmol Cmol}^{-1} \text{h}^{-1}$), as explained in the appendix.

The results are shown in Figure 3.3. The rates calculated for continuous illumination represent a continuous rate; whereas the rate for intermittent light is the rate per milliseconds of flash time, since reducing equivalents are only produced during the flash.

At a flash frequency of 5 Hz the reducing equivalent production rate was 2.5 times greater and at 100 Hz it was 5 times greater than the rate at maximum growth under continuous illumination of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$, where it was $0.08 \mu\text{mol}_H\text{-Cmol}^{-1} \text{ms}^{-1}$.

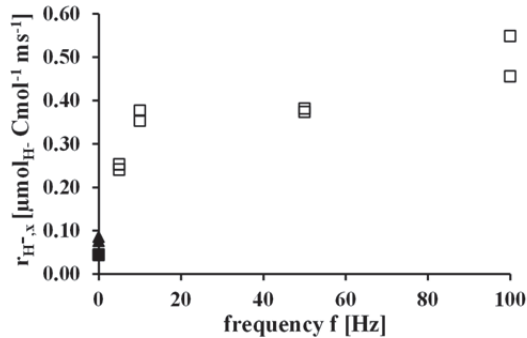


Figure 3.3 Reducing equivalent (H) production rate versus flash frequency, intermittent illumination: \square , continuous illumination: \blacktriangle ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$), \blacksquare ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$)

The increase in the production rate of reducing equivalents (i.e. equivalent to the rate of linear photosynthetic electron transport) may be the key to explain light integration. Under continuous illumination with the time-averaged PFD of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ linear electron transport proceeded at a base rate and is consumed at the same rate by the dark reaction. Reducing equivalents can only be processed at a maximum rate which is determined by the maximum specific growth rate. In flashing light the production and consumption rates of reducing equivalents were imbalanced and as the calculations showed the production rate of reducing equivalents was at least 2.5 fold greater in

flashing light than under continuous illumination. Since the maximum processing rate is determined by the maximum growth rate not all of the produced reducing equivalents can be used directly during the flash. In order to reach full light integration the rest must have been stored in an electron pool and made available during the dark part of the L/D cycle.

The nature of the electron pool is not known. It is probably a combination of different compounds (NADPH, plastoquinol, or reduced carbon compounds from the Calvin-cycle). During the dark phase of the cycle the photosynthetic electron transport chain had time to equilibrate and increase the efficiency of photon processing during the next flash. However, the size of the electron pool seems only sufficient to achieve full light integration at flash times around 1 ms, whereas during longer flash times the pool will overflow during the light phase resulting in a loss of photosynthetic efficiency and a decrease in the biomass yield on light.

During all flashing light experiments an equal amount of photons was absorbed, which is indicated by the constant absorptive surface. However, big differences in biomass yields were observed. At lower flash frequencies the exposure to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ during the flash was long enough to over-saturate the photosystem which caused a decrease in efficiency. Full light integration will be difficult to achieve in real PBR systems because the maximum flash frequency typically observed in a PBR system will not exceed $\approx 25 \text{ Hz}$ as shown in measurements and CFD simulations (Luo et al., 2003; Pernernochta and Posten, 2007).

This would also explain the results of Zijffers (2009), Zijffers et al. (2010) and Kliphuis et al. (2010a), since in their systems the photosynthetic efficiency could not be significantly increased with change in gas rate, light path and mixing speed. However, even at the lowest flash frequency (5 Hz) tested in this study the biomass yield on light energy was higher than for the estimated (but not experimentally measured) value in continuous light at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (flash intensity).

Although full light integration was only observed at 100 Hz the flashing light effect could still have a measurable and beneficial effect on the efficiency of short light path photobioreactors. In PBRs with optical paths in the order of two to four centimeters, average light/dark fluctuations can be as high as $\approx 25 \text{ Hz}$ (Luo et al., 2003; Pernernochta and Posten, 2007). As shown in our study, under these conditions still a significant amount of electrons could be stored in the photic zone and utilized in the dark

zone, leading to more efficient use of light energy. To provide better guidelines for photobioreactor design and operation, experiments with a broadened range of light/dark ratios and incident PFDs are still necessary to be able to set up a predictive model for microalgae growth under fluctuating light. In addition to the biological response, photobioreactor hydrodynamics must be characterized to be able to estimate the light history of individual cells.

Conclusions

For the first time the biomass yield on light energy was determined for green microalgae acclimated to sub-second flashing light regimes in turbidostat cultures. In order to evaluate these results, reference experiments under continuous illumination were done. It was found that very short flash times (≈ 1 ms) are necessary to achieve full light integration. Nevertheless, even at the longer flash times (lower frequencies, 5 Hz and 10 Hz) tested, the biomass yield on light energy was higher than for the estimated value for continuous illumination with an over-saturating photon flux density. The increased yields under flashing light can be explained by enhanced rates of reducing equivalent production during the flash in combination with a storage pool of reducing equivalents which is accessible for microalgae growth during the dark period of one L/D cycle.

Acknowledgements

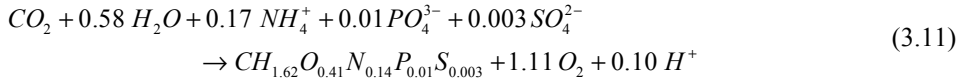
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Appendix

Calculation of production rate of reducing equivalents

The production rate of reducing equivalents was calculated based on linear photosynthetic electron transport, the stoichiometry for growth on ammonium and the measured, specific growth rates (μ , $Cmol\ Cmol^{-1}\ h^{-1}$).

The consumption of 1 mol CO_2 results in the formation of 1 Cmol biomass and the net production of 1.11 mol O_2 based on the measured elemental composition (Kliphuis, 2010b) of *Chlamydomonas reinhardtii* ($Y_{O_2/Cmol} = 1.11$, equation (3.11)).



Net oxygen production (r_{O_2} , equation (3.12)) is only possible based on linear electron transport. In this case the final electron acceptor is $NADP^+$.

$$r_{O_2} = \mu \cdot Y_{O_2/Cmol} \quad (3.12)$$

For the formation of 1 mol of oxygen, four electrons have to be translocated and these are initially used to produce 2 mol reducing equivalent (H^-) in form of $NADPH/H^+$ ($Y_{H^-/O_2} = 2$, equation (3.13)) and later to fixate and reduce carbon dioxide. To convert from $mol_{H^-}\ Cmol^{-1}\ h^{-1}$ into $\mu mol_{H^-}\ Cmol^{-1}\ ms^{-1}$ a factor of 3.6 is introduced in equation (3.13)

$$r_{H^-} = r_{O_2} \cdot Y_{H^-/O_2} \cdot 3.6^{-1} \quad (3.13)$$

The electron production rate in equation (3.13) is based on continuous illumination. For flashing light the reducing equivalents have to be produced during the light time (t_l):

$$r_{H^-}^{flash} = r_{H^-} \cdot \phi^{-1} \quad (3.14)$$

Relative light distribution

Figure 3.4 shows the relative light distribution across the illuminated surface. The photon flux density at any given position was normalized to the average photon flux density.

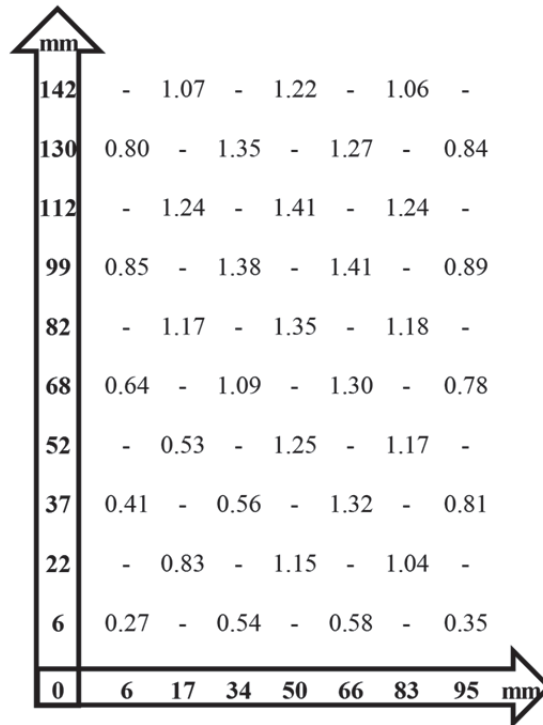


Figure 3.4 Relative photon flux density distribution across the illuminated surface. The origin of the coordinate system is located in the bottom left corner of the photobioreactor cuvette.

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Chapter 4

Photosynthetic efficiency and oxygen evolution of *Chlamydomonas reinhardtii* under continuous and flashing light

Abstract

As a result of mixing and light attenuation in a photobioreactor, microalgae experience light/dark (L/D) cycles that can enhance photobioreactor efficiency. One parameter which characterizes L/D cycles is the duty cycle; it determines the time fraction algae spend in the light.

The objective of this study was to determine the influence of different duty cycles on oxygen yield on absorbed light energy and photosynthetic oxygen evolution. Net oxygen evolution of *Chlamydomonas reinhardtii* was measured for four duty cycles (0.05, 0.1, 0.2 and 0.5) in a biological oxygen monitor. Over-saturating light flashes were applied in a square-wave fashion with four flash frequencies (5, 10, 50 and 100 Hz). Algae were pre-cultivated in a turbidostat and acclimated to a low photon flux density (PFD).

A photosynthesis-irradiance curve was measured under continuous illumination and used to calculate the net oxygen yield, which was maximal between a PFD of 100 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Net oxygen yield under flashing light was duty cycle dependent: the highest yield was observed at a duty cycle of 0.1 (i.e. time-average PFD of 115 $\mu\text{mol m}^{-2} \text{s}^{-1}$). At lower duty cycles maintenance respiration reduced net oxygen yield. At higher duty cycles photon absorption rate exceeded the maximal photon utilization rate, and, as a result, surplus light energy was dissipated which lead to a reduction in net oxygen yield. This behavior was identical with the observation under continuous light.

Based on these data the optimal balance between oxygen yield and production rate can be determined to maximize photobioreactor productivity.

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Introduction

High photobioreactor (PBR) productivity is crucial to microalgae feed stock production and depends, amongst others, on a high biomass yield on light energy.

If a PBR is exposed to (sun) light then light is attenuated along the PBR depth as a function of algae concentration and pigmentation. A high biomass concentration results in an illuminated zone with net photosynthesis and a dark zone with possible respiration. As a result of mixing algae experience, therefore, flashing light or light/dark (L/D) cycles. These mixing-induced L/D cycles are suggested to enhance PBR efficiency (Degen et al., 2001; Hu and Richmond, 1996; Richmond, 1996).

Algae growing at (over-) saturating incident photon flux densities show a low photosynthetic efficiency. But, if algae are grown under light limiting conditions then photosynthetic efficiency is high (Tredici, 2009). Photosynthetic efficiency can be expressed in different ways: biomass yield on light energy (i.e. gram of biomass produced per mol of PAR photons absorbed) or quantum yield of oxygen evolution (i.e. mol of oxygen produced per mol of PAR photons absorbed). With the knowledge of growth stoichiometry oxygen yield can be converted to biomass yield and used to evaluate PBR performance.

The effect of a dynamic light regime on biomass yield can be assessed with the concept of light integration (Phillips and Myers, 1954; Terry, 1986). Under full light integration algae do not respond to the actual photon flux density (PFD) during the flash of an L/D cycle, but to the lower, time-averaged PFD of the whole cycle (flash + dark time). In other words, biomass yield will be comparable with cultivation under continuous and limiting PFD. Opposite to full light integration algae can respond directly to the PFD during the flash and respire during the dark part of the L/D cycle. This behavior can be called growth integration and is not favorable because it leads to low biomass yields.

Photosynthetic rate measurements based on oxygen evolution have shown that sub-second L/D cycles are necessary to achieve (full) light integration (Kok and Burlew, 1953; Matthijs et al., 1996; Nedbal et al., 1996; Terry, 1986). These results are supported by the measurement of specific growth rate under flashing light (Phillips and Myers, 1954; Rosello-Sastre, 2010; Vejrazka et al., 2011; Xue et al., 2011). The major influence of L/D cycles on photosynthetic rate was found with over-saturating, incident PFDs during the flash (Vejrazka et al. 2011; Xue et al., 2011). But, most studies presented only results about photosynthetic rates and did not include photosynthetic efficiency, which is an important parameter because it incorporates the absorption cross section and the

actual amount of light absorbed by the algae. As a consequence, the full impact of L/D cycles on PBR productivity is still not fully understood.

One parameter which characterizes and influences the amount of light integration is the duty cycle: it determines the time fraction algae spend in the light during one full L/D cycle. In a PBR the duty cycle is dependent on the biomass concentration. An increase in biomass concentration will lead to an increase in a dark zone and thus a decrease in duty cycle, which might affect algae growth and productivity. The objective of our study was to determine the influence of different duty cycles on light integration.

The degree of light integration was assessed both in terms of photosynthetic rate and oxygen yield. Oxygen evolution rates in different (dynamic) light regimes were measured in a newly developed biological oxygen monitor.

To be able to properly discuss the results from flashing light we measured net and gross oxygen production rates in continuous light and determined the corresponding oxygen yields (net and gross). The net oxygen production rate in continuous light could be described by a hyperbolic tangent model including maintenance respiration. This paper provides a systematic investigation on the effect of duty cycle in addition to flash frequency on oxygen production and more important photosynthetic efficiency.

Materials and Methods

Organism and medium

Chlamydomonas reinhardtii CC-124 wild type mt- 137c was obtained from the Chlamydomonas culture collection at Duke University (now Chlamydomonas resource center). The microalgae were cultivated in a Sueka high salt (HS) based liquid medium, composed of (amounts in g L^{-1}): NH_4Cl , 1.450; KH_2PO_4 , 0.720; K_2HPO_4 , 1.440; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.280; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.057; $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 0.067 and 10 mL L^{-1} of a 100 times concentrated Hutner's trace elements solution (Hutner et al., 1950). Axenic cultures were maintained in shake flasks placed on an orbital shake incubator at pH 7, 25 °C, air enriched with 2.5 %_{v/v} CO_2 , and 60 – 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ continuous illumination for at least one week before use as photobioreactor (PBR) inoculum.

Photobioreactor set-up

The PBR system (Photobioreactor FMT 150, Nedbal et al., 2008) was purchased from Photon System Instruments (PSI, Brno, Czech Republic) and equipped with a red LED panel peaking at 630 nm.

The PBR was operated as a turbidostat (axenic) with an incident photon flux density (PFD) of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$. The PFD leaving the PBR was set to $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ to obtain low light acclimated algae because Vejrazka et al. (2011) showed that algae grown under flashing light conditions with a low time-averaged PFD ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) were acclimated to a low PFD.

Temperature was regulated at $25 \text{ }^\circ\text{C}$ and the culture was mixed by sparging air at a flow rate of $1 \text{ L}_{\text{gas}} \text{ L}_{\text{culture}}^{-1} \text{ min}^{-1}$. The pH was maintained at 7.0 via pulse-wise CO_2 addition to the air stream. For this reason, the PBR medium was enriched with 0.420 g L^{-1} of NaHCO_3 . After a steady state was reached algae were used for oxygen evolution measurements. In this way it was assured that algae were always acclimated to the same light regime. The general operation procedure for the PBR is described in Vejrazka et al. (2011).

Biological oxygen monitor setup

We designed a biological oxygen monitor (BOM) for the oxygen evolution measurements. The BOM and the light modules (including control) were manufactured by the 'Ontwikkelwerkplaats' of Wageningen University. The BOM consisted of three chambers: two water jackets, one measurement chamber (see Figure 4.1). The cylindrical measurement chamber had a diameter of 31 mm and a light path of 15 mm (liquid volume: 12.3 mL). It had three ports: one to inject the sample, one to release gas and one to house the oxygen probe holder. Each side of the measurement chamber was coupled to a water jacket, which was connected to a water bath. The 4 mm thick windows of the BOM were made from the transparent polycarbonate resin Lexan.

The BOM setup was placed on a magnetic stirring plate to mix the algae suspension with a tumble stir disc (VP 719F-2, V&P Scientific, Inc., San Diego, USA).

The BOM was illuminated from each side with one round LED module emitting red light peaking at 620 nm. Each LED module was equipped with 19 Yoldal (YE-R5S15N) LEDs. The LED current was set to 20 mA and the brightness was controlled by pulse width modulation at a frequency of 10 kHz. The flash time and dark time of the LED modules could be set in 1 ms steps from 1 ms to 9999 ms.

The dissolved oxygen concentration was measured with an optical oxygen microsensors (IMP-PS1-L5-LIC0-BGF10-TF-OIW, Presens, Regensburg, Germany). The measurement tip of the sensor was flat broken ($\varnothing \approx 140 \mu\text{m}$) and coated with an optical insulation to prevent influence of ambient light and chlorophyll fluorescence on the

measurement. The signal was processed via a transmitter (Microx TX3, Presens, Regensburg, Germany) and recorded on a PC with the program Microx TX3 for PSt1 (Ver. 6.02, Presens, Regensburg, Germany).

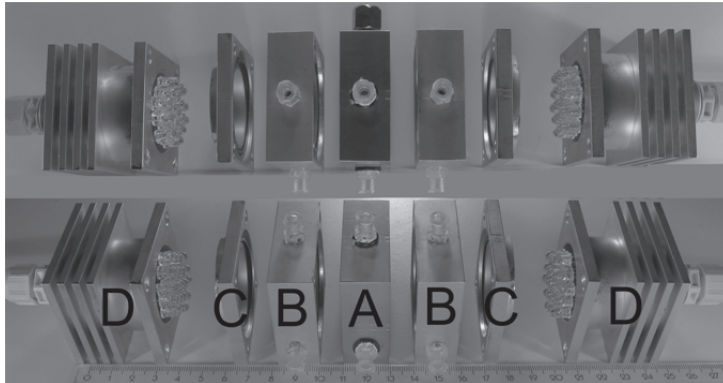


Figure 4.1 Biological oxygen monitor. A: measurement chamber, B: water jacket, C: end piece, D: LED module

Biological oxygen monitor operating procedure

All measurements and calibrations were done with an active water circulation through the water jacket to control the temperature at 25 °C.

The oxygen probe was calibrated with a two point calibration (0 %, 100 % air-saturation) before the oxygen evolution measurement. The probe was calibrated inside the measurement chamber which was filled with medium (without NaHCO_3) and bubbled with air (100 % air-saturation) or pure nitrogen (0 % air-saturation).

Algae samples were taken during steady-state operation of the PBR for oxygen evolution measurements. In this way it was assured that the algae were always acclimated to the same low light conditions. The PBR was mixed with pure nitrogen instead of air for 7 min to reduce the dissolved oxygen concentration in the algae suspension to a level between 20 % and 30 % before a sample (14 mL) was transferred to the BOM.

After sample injection into the BOM algae were dark-acclimated for 5 min and then dark respiration (i.e. maintenance respiration) was measured for 10 min. Then, the light was turned on and oxygen production was measured for 3 to 5 min. Next, lights were turned off and light respiration was measured for 5 min. Only the first 3 min of data were used to calculate the oxygen consumption rate. After the light respiration measurement, light was turned on again and the whole procedure was repeated at another PFD or duty cycle. In flashing light no light respiration was measured.

Preliminary experiments showed that one sample could not be used for more than 1 hour total measurement time, or above a dissolved oxygen concentration of 130 %. Otherwise, oxygen production rates under the same conditions changed. Each setting was measured at least three times. Each measurement block (maximally 1 h of measurement) was designed as such that the PFD (time-averaged PFD in case of flashing light) was increased from first to last setting to prevent measuring oxygen evolution of photodamaged algae at low PFDs .

The dissolved oxygen concentration was recorded in one second intervals and expressed as percent (%) air saturation. The conversion to $\mu\text{mol L}^{-1}$ was done based on Henry's law, thus correcting the maximum solubility of oxygen in water for the temperature influence: 100 % air saturation equals a dissolved oxygen concentration of $258 \mu\text{mol L}^{-1}$ at $25 \text{ }^\circ\text{C}$. The oxygen production rate (P_{O_2} , $\mu\text{mol}_{O_2} \text{g}^{-1} \text{s}^{-1}$) is the slope of dissolved oxygen concentration versus time divided by the biomass concentration (c_x , g L^{-1}).

Light regime biological oxygen monitor

The PFDs for continuous light can be found in Table 4.1.

Table 4.1 PFD settings for PI-curve measurements

	PFD_L	
	$\mu\text{mol m}^{-2} \text{s}^{-1}$	
	LED1	LED2
1	10	-
2	30	-
3	42	-
4	52	-
5	85	-
6	95	-
7	182	-
8	424	-
9	536	-
10	875	-
11	536	543

The highest photon flux density (PFD) in continuous light was $1079 \mu\text{mol m}^{-2} \text{s}^{-2}$, which was also set as the flash PFD. This PFD was supplied by two LED modules: each LED module providing half the target PFD from each side of the measurement chamber. Net oxygen production was compared for different PFDs provided either from one LED module or half the target PFD from two modules (Figure 4.2). Data shows that the

oxygen production measured with either one or two LED modules are linearly correlated with a zero offset. Therefore, the PFD can be supplied either from one or from two sides and there is no influence on the net oxygen production rate.

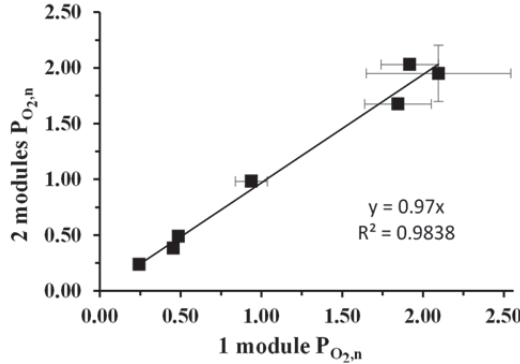


Figure 4.2 Net oxygen production rates for two LED modules versus one LED module at different photon flux densities. Error bars represent the standard deviation from two measurement

The flashing light regime was defined by the length of the square-wave flash (t_f , ms), dark time (t_d , ms), cycle time ($t_c = t_f + t_d$, ms), duty cycle ($\phi = t_f/t_c$, -), and flash frequency ($f = 1000/t_c$, Hz). The flash photon flux density (PFD_L , $\mu\text{mol m}^{-2} \text{s}^{-1}$) was provided by two sides and set to a total of $1079 \mu\text{mol m}^{-2} \text{s}^{-1}$ (LED1: $536 \mu\text{mol m}^{-2} \text{s}^{-1}$, LED2: $543 \mu\text{mol m}^{-2} \text{s}^{-1}$).

In flashing light four different duty cycles (0.05, 0.1, 0.2 and 0.5) and four different flash frequencies (5, 10, 50 and 100 Hz) were tested.

Photosynthesis-irradiance curve and respiration

The response to continuous light (PI-curve) is best described by the hyperbolic tangent model (Jassby and Platt, 1976; equation (4.1)): with the initial slope of the curve (α , $\mu\text{mol O}_2 \text{ m}^{-2} \mu\text{mol}_{ph}^{-1} \text{ g}^{-1}$) and the maximal net oxygen production rate ($P_{O_2,n}^{\max}$, $\mu\text{mol O}_2 \text{ s}^{-1} \text{ g}^{-1}$). To account for oxygen consumption at PFDs below the compensation point maintenance respiration (R_{ms} , $\mu\text{mol O}_2 \text{ g}^{-1} \text{ s}^{-1}$) was introduced in equation (4.1).

$$P_{O_2,n} = \left((P_{O_2,n}^{\max} + R_{ms}) \cdot \tanh \left(\frac{\alpha \cdot PFD}{(P_{O_2,n}^{\max} + R_{ms})} \right) \right) - R_{ms} \quad (4.1)$$

Algae respiration rate (R , $\mu\text{mol O}_2 \text{ g}^{-1} \text{ s}^{-1}$) can be determined with equation (4.2), with maintenance respiration (R_{ms} , $\mu\text{mol O}_2 \text{ g}^{-1} \text{ s}^{-1}$), net oxygen production rate ($P_{O_2,n}$, $\mu\text{mol O}_2 \text{ g}^{-1} \text{ s}^{-1}$) and a linear correlation factor ($c_R = 0.162$), which describes

growth-associated respiratory activity. Maintenance respiration rate and linear correlation factor were determined by plotting the measured light respiration (i.e. post illuminated respiration, Kliphuis et al., 2011b) versus the net oxygen production rate (Figure 4.4a). Additionally, gross oxygen production rate ($P_{O_2,g}, \mu mol O_2 g^{-1} s^{-1}$) can be defined as the sum of respiration and net oxygen production rate (equation (4.3)).

$$R = R_{ms} + c_R \cdot P_{O_2,n} \quad (4.2)$$

$$P_{O_2,g} = P_{O_2,n} + R \quad (4.3)$$

Light measurement continuous light

The relative, spatial distribution of the incident PFD ($PF D_L^{sp}(i)$, $\mu mol m^{-2} s^{-1}$) was measured at nine different points (i) inside the measurement chamber of the BOM at the light exposed surface and averaged into one relative PFD ($PF D_L^{sp}$, $\mu mol m^{-2} s^{-1}$). This measurement was done with an Avantes setup, which is a fiber optic CCD based spectroradiometer (AvaSpec-2048 detector, Fiber FC-IR100-1-ME, 400-700nm, Avantes, Eerbeek, The Netherlands). By dividing the PFD of position 9 ($PF D_L^{sp}(9)$, center position) with the average PFD ($PF D_L^{sp}$) a correlation factor cf was calculated.

The absolute PFD ($PF D_L(9)$) was measured at position 9 (center position) with a LiCor 190-SA 2π PAR (400-700 nm) quantum sensor (LiCor, Lincoln, Nebraska). The absolute, average PFD ($PF D_L$) was calculated by dividing the absolute PFD at position 9 ($PF D_L(9)$) with the correlation factor cf. In case of two LED modules the total incident PFD was the sum of the PFD of each module.

The light transmission ($T(i)$, equation (4.4)) was measured at three different positions behind the measurement chamber with the Avantes setup. First a blank PFD ($PF D_{L,blank}^{sp}(i)$) was measured while the chamber was filled with medium, stirred and illuminated. Second, an algae sample was injected and the PFD ($PF D_{L,algae}^{sp}(i)$) was measured again while the suspension was stirred and illuminated.

$$T(i) = \frac{PF D_{L,algae}^{sp}(i)}{PF D_{L,blank}^{sp}(i)} \quad (4.4)$$

In case of two modules the light transmission was measured for each module.

Light measurement in flashing light

If LEDs are flashed with different frequencies LED temperature and, as a result, LED light output can vary. For this reason we measured the relative, time-averaged PFD ($PFD_{L/D}$) for each flashing light regime with the Avantes setup at position 9.

The integration time of the Avantes setup was set to 2 ms and a total of 2500 measurements were averaged to obtain a stable signal in flashing light. First, a relative reference PFD ($PFD_L^{sp}(9)$) was measured for each LED module. For this measurement the module was set to the flash intensity (continuous illumination). Second, a relative PFD ($PFD_{L/D}^{sp}(9)$) was measured for each flashing light setting. The absolute, time-averaged PFD ($PFD_{L/D}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$) was then calculated according to equation (4.5).

$$PFD_{L/D} = PFD_L \cdot \frac{PFD_{L/D}^{sp}(9)}{PFD_L^{sp}(9)} \quad (4.5)$$

This measurement was done for each module and the total PFD was the sum of both measurements.

Dry weight determination

For dry weight measurement Whatman GF/F glass microfiber filters ($\text{\O} 55 \text{ mm}$, pore size = $0.7 \mu\text{m}$) were dried (24 h, $95 \text{ }^\circ\text{C}$), cooled down to room temperature in a desiccator ($> 2\text{h}$) and weighed. The sample ($< 5 \text{ mg}$ dry weight) was filtrated with a constant vacuum (0.44 bar, absolute), washed three times with MilliQ water, dried (24 hours, $95 \text{ }^\circ\text{C}$), cooled down in a desiccator ($> 2\text{h}$) and weighed again. The weight difference between empty and algae containing filter was the dry weight. Each sample was measured in triplicate.

Oxygen yield on absorbed light energy

The biomass specific photon absorption rate ($r_{ph,abs}$, $\mu\text{mol}_{ph} \text{g}^{-1} \text{s}^{-1}$) was calculated based on equation (4.6):

$$r_{ph,abs} = PFD_L \cdot A_{BOM} \cdot \left(1 - \left(\sum_{i=1}^3 T(i) \right) \cdot 3^{-1} \right) \cdot (c_x \cdot V_{BOM})^{-1} \quad (4.6)$$

With:

- incident PFD (PFD_L , $\mu\text{mol}_{ph} \text{m}^{-2} \text{s}^{-1}$, continuous light)
- the illuminated surface of the BOM ($A_{BOM} = 7.55 \cdot 10^{-4} \text{m}^2$)

- the light transmission ($T(i), -$)
- the biomass concentration ($c_x, g m^{-3}$)
- the volume of the BOM ($V_{BOM} = 1.2 \cdot 10^{-5} m^3$).

If two modules were used then the photon absorption rate was calculated for each module separately and the total photon absorption rate was the sum of both calculations.

The photon absorption rate for continuous light showed a linear correlation with the incident PFD (Figure 4.3). This linear correlation was used to determine the photon absorption rate for flashing light based on the measured, time-averaged PFDs.

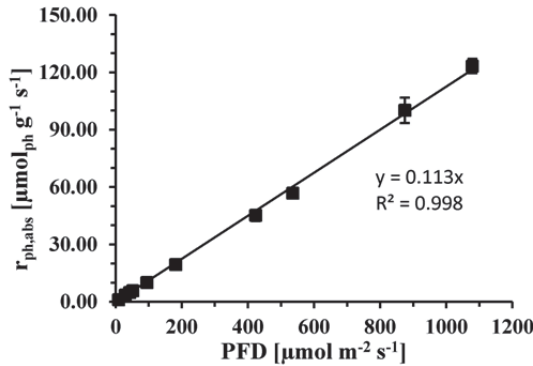


Figure 4.3 Biomass specific photon absorption rate versus incident photon flux density in continuous light

The net (n) oxygen yield ($Y_{O_2/ph,n}, mol_{O_2} mol_{ph}^{-1}$) or gross (g) oxygen yield ($Y_{O_2/ph,g}, mol_{O_2} mol_{ph}^{-1}$) on absorbed photons is the quotient of net (n) oxygen production rate ($P_{O_2,n}, \mu mol_{O_2} g^{-1} s^{-1}$) or gross (g) oxygen production rate ($P_{O_2,g}, \mu mol_{O_2} g^{-1} s^{-1}$) and biomass specific light absorption rate ($r_{ph,abs}$, equation (4.7)).

$$Y_{O_2/ph,z} = \frac{P_{O_2,z}}{r_{ph,abs}}, z \in [n, g] \quad (4.7)$$

Results

The first 200 hours of photobioreactor (PBR) operation were necessary to reach steady-state; followed by a steady state operation of about 800 hours. The average biomass specific growth rate during the steady state was constant at $0.059 \pm 0.002 h^{-1}$ and a biomass concentration of $0.095 \pm 0.005 g L^{-1}$. The average dry weight specific absorption coefficient (see Vejrazka et al. 2011) was $0.21 \pm 0.01 m^2 g^{-1}$ (determined after 250 h, 450 h and 800 h of steady state). The resulting biomass yield on absorbed light

energy was $0.99 \pm 0.08 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$. Based on growth stoichiometry and given elemental composition of the biomass (Vejrazka et al., 2011) the biomass yield could be recalculated into a net oxygen yield on absorbed light energy: $0.046 \pm 0.004 \text{ mol}_{\text{O}_2} \text{ mol}_{\text{ph}}^{-1}$.

Photosynthesis-Irradiance curve in continuous light

To evaluate the effect of flashing light on oxygen production rate or oxygen yield on absorbed light energy, reference points under continuous illumination are necessary. Therefore, we measured a photosynthesis-irradiance (PI) curve of *C. reinhardtii* under continuous illumination.

The hyperbolic tangent model fits the measured PI-curve well (Figure 4.4b). The net oxygen production rate was zero at a PFD of $11 \mu\text{mol m}^{-2} \text{ s}^{-1}$ which is similar to the compensation point ($10 \mu\text{mol m}^{-2} \text{ s}^{-1}$) determined by Takache et al. (2010). After the compensation point the net oxygen production rate increases linearly with increase in PFD until a PFD of about $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$. With further increase in PFD the net oxygen production rate becomes saturated and reaches its maximum ($1.87 \pm 0.20 \mu\text{mol}_{\text{O}_2} \text{ s}^{-1} \text{ g}^{-1}$) at about $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$. This maximal net oxygen production rate can be converted to a maximal specific growth rate based on the growth stoichiometry and elemental composition shown in Vejrazka et al. (2011). The maximal growth rate results in $0.14 \pm 0.02 \text{ h}^{-1}$ which is comparable to the measured value in Vejrazka et al. (2011).

The light respiration rate is linearly correlated with the net oxygen production rate (Figure 4.4a) which is in agreement with Falkowski et al. (1985) and Geider and Osborne (1989). The intercept with the y-axis (Figure 4.4a) results in the maintenance respiration rate (R_{ms}) which was $0.11 \mu\text{mol}_{\text{O}_2} \text{ s}^{-1} \text{ g}^{-1}$. This maintenance respiration rate is similar to the rate ($0.10 \mu\text{mol}_{\text{O}_2} \text{ s}^{-1} \text{ g}^{-1}$) Kliphuis et al. (2011b) determined for *Chlorella sorokiniana* but differs from the rate ($0.15 \mu\text{mol}_{\text{O}_2} \text{ s}^{-1} \text{ g}^{-1}$) Kliphuis et al. (2011a) calculated for *Chlamydomonas reinhardtii* based on a metabolic growth model. Although, the maintenance respiration determined by Kliphuis et al. (2011a) is not exactly the same as in this study, it is in the same range. The difference might be due to the two completely different methods used to determine this parameter.

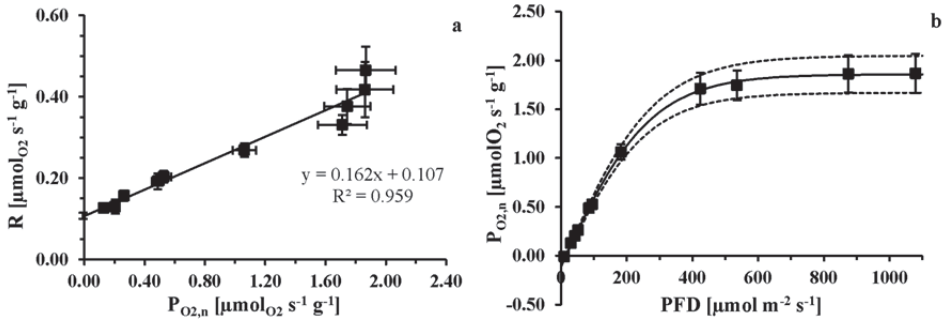


Figure 4.4 a) light respiration vs. net oxygen production rate, ■ measured values, — linear fit ($C_R = 0.162, R_{ms} = 0.107 \mu\text{mol}_{\text{O}_2} \text{s}^{-1} \text{g}^{-1}$); b) net oxygen production rate vs. incident photon flux density ■ measured values, — and --- fit based on hyperbolic tangent model (equation (4.1)), model parameters: $P_{\text{O}_2,n}^{\text{max}} = 1.86 \pm 0.19 \mu\text{mol}_{\text{O}_2} \text{s}^{-1} \text{g}^{-1}$, $\alpha = 0.0073 \pm 0.0004 \mu\text{mol}_{\text{O}_2} \text{m}^2 \mu\text{mol}_{\text{ph}}^{-1} \text{g}^{-1}$, $R_{ms} = 0.107 \mu\text{mol}_{\text{O}_2} \text{s}^{-1} \text{g}^{-1}$, error bars represent the standard deviation of at least three measurements.

Based on the photon absorption rate and the oxygen production rate (gross rate and net rate) oxygen yield on absorbed photons (gross yield and net yield) was calculated (Figure 4.5). Net oxygen yield increases with increase in PFD and reaches its maximum between $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Further increase in PFD results in a decrease in net oxygen yield. The low yield at low PFDs ($<100 \mu\text{mol m}^{-2} \text{s}^{-1}$) can be explained by the influence of maintenance respiration (i.e. dark respiration): the lower the oxygen production resulting from photosynthesis the greater is the relative part which has to be used for maintenance respiration. Whereas, at high PFDs ($>500 \mu\text{mol m}^{-2} \text{s}^{-1}$) photon absorption rate exceeds the maximal photon utilization rate, and, as a result, the surplus light energy must be dissipated.

The influence of respiration is best demonstrated by calculating the gross oxygen yield (Figure 4.5, open squares) and comparing it to the net yield. The gross yield reflects the actual rate of linear electron transport by photosynthesis in the chloroplast. Data in Figure 4.5 shows that this yield is slightly decreasing until a PFD of about $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and then it decreases more rapidly. This rapid decrease indicates that more photons are being absorbed than can be used and excess energy must be dissipated. The gross yield at $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ shows a big standard deviation because measurable net oxygen production rate was around zero.

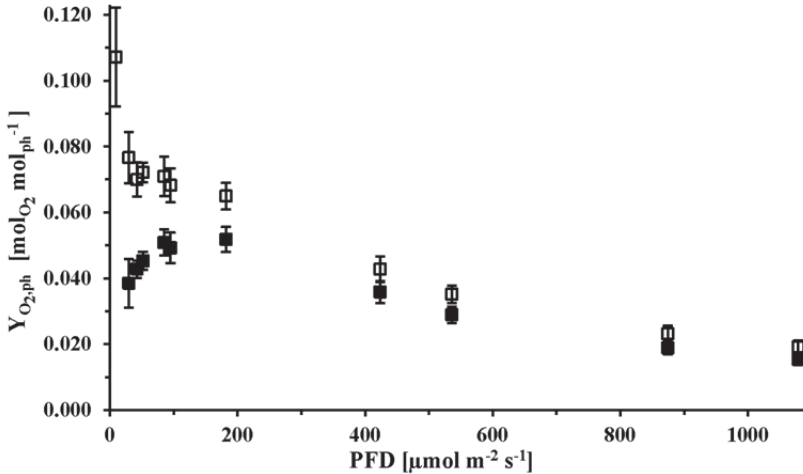


Figure 4.5 Oxygen yield on absorbed photons vs. incident photon flux density, ■ net yield □ gross yield (i.e. direct yield of photosystem II)

Flashing light

In total four different duty cycles and four different flash frequencies were tested. The results of the measured net oxygen production rates ($P_{O_2,n,L/D}$) are summarized in Table 4.2. If only one duty cycle is considered then the oxygen production rate increases with increase in flash frequency, which is in agreement with other studies (Phillips and Myers 1954, Terry 1986, Vejrazka et al. 2011, Xue et al. 2011). Furthermore, if only a specific flash frequency is considered then the net oxygen production rate increases with increase in duty cycle, which was expected because an increase in duty cycle coincides with an increase in time-averaged PFD.

To evaluate the amount of light integration in flashing light the net oxygen production rate for continuous illumination with the time-averaged PFD has to be calculated with the hyperbolic tangent model shown in equation (4.1).

For this calculation it is necessary to know exactly the measured, time-averaged PFD of one L/D cycle (Table 4.2, $PFD_{L/D}$). Measurements showed that especially at a frequency of 50 Hz and 100 Hz the time-averaged PFD increased in comparison to 5 Hz and 10 Hz. But, also at the lower flash frequencies the time-average PFD was higher than expected based on duty cycle and flash PFD. This increase in time-averaged PFD was caused by the LEDs heating up less while flashing compared to continuous light. At lower temperatures LEDs convert more electricity into light.

As a result of the different, time-averaged PFDs, the calculated net oxygen production rate for equivalent continuous illumination is not the same for one duty cycle, which was accounted for in the calculation of the reference oxygen production rates based on light integration (Table 4.2, $P_{O_2,n}$). A comparison of these rates with the measured rates from flashing light shows that the rate in flashing light does not exceed the rate in continuous light (based on light integration). These results are in agreement with the hypothesis of Rabinowitch (1956) that the photosynthetic rate in flashing light does not exceed the one under continuous illumination with the same time-averaged PFD.

Table 4.2 Results of oxygen evolution measurements in flashing light, values in brackets are standard deviations for triplicate measurements.

Φ	f	t_l	t_d	$PFD_{L/D}$	$P_{O_2,n,L/D}$	$P_{O_2,n}$
-	Hz	ms	ms	$\mu mol m^{-2} s^{-1}$	$\mu mol_{O_2} s^{-1} g^{-1}$	$\mu mol_{O_2} s^{-1} g^{-1}$
0.05	5	10	190	58	0.22 (0.02)	0.31 (0.03)
	10	5	95	58	0.27 (0.01)	0.31 (0.03)
	50	1	19	67	0.37 (0.01)	0.37 (0.03)
0.1	5	20	180	113	0.46 (0.02)	0.67 (0.05)
	10	10	90	114	0.55 (0.05)	0.68 (0.05)
	50	2	18	118	0.70 (0.04)	0.70 (0.05)
	100	1	9	132	0.80 (0.01)	0.78 (0.06)
0.2	5	40	160	227	0.82 (0.03)	1.24 (0.10)
	10	20	80	227	0.87 (0.07)	1.24 (0.10)
	50	4	16	232	1.02 (0.07)	1.26 (0.10)
	100	2	8	238	1.36 (0.02)	1.28 (0.10)
0.5	5	100	100	559	1.55 (0.04)	1.80 (0.17)
	10	50	50	559	1.45 (0.04)	1.80 (0.17)
	50	10	10	557	1.59 (0.15)	1.79 (0.17)
	100	5	5	561	1.66 (0.01)	1.80 (0.17)

To properly assess the amount of light integration it is possible to calculate an integration factor (IF, equation (4.8)) similar to the one proposed by Terry (1986). This integration factor (IF) is based on the measured net oxygen production rate in flashing light ($P_{O_2,n,L/D}$, Table 4.2), net oxygen production rate based on growth integration ($P_{O_2,n,gi}$, equation (4.9)) and net oxygen production rate based on light integration ($P_{O_2,n}$, Table 4.2). Oxygen production for growth integration (equation (4.9)) is based on the assumption that algae photosynthesis during the flash is equivalent to the rate under continuous illumination with the flash PFD. Furthermore, it is assumed that maintenance respiration occurs during the dark part of the cycle.

$$IF = \frac{P_{O_2,n,L/D} - P_{O_2,n,gi}}{P_{O_2,n} - P_{O_2,n,gi}} \quad (4.8)$$

$$P_{O_2,n,gi} = \phi \cdot P_{O_2,n} - (1-\phi) \cdot R_{ms} \quad (4.9)$$

The results of the integration factor are shown in Figure 4.6: an integration factor of one is equal to full light integration and an integration factor of zero is equal to growth integration. During all tested L/D cycles the rate of photosynthesis was above the rate expected for solely growth integration which is in agreement with observations that sub-second L/D cycles are necessary to achieve (partial) light integration.

In general data shows that the amount of light integration depends on the duty cycle but also on flash frequency. A flash frequency of 100 Hz resulted in full light integration except for the duty cycle of 0.5. At this duty cycle full light integration was not achieved at any frequency tested. A frequency of 50 Hz led to full light integration at a duty cycle of 0.05 and 0.1 but not at 0.2 as observed for 100 Hz. Frequencies below 50 Hz did not result in full light integration at any duty cycle but resulted in partial light integration, whereas the amount of light integration decreased with increase in duty cycle and decrease in flash frequency. An exception can be found for the duty cycle of 0.5 where light integration increased again in comparison to a duty cycle of 0.2 and 0.1.

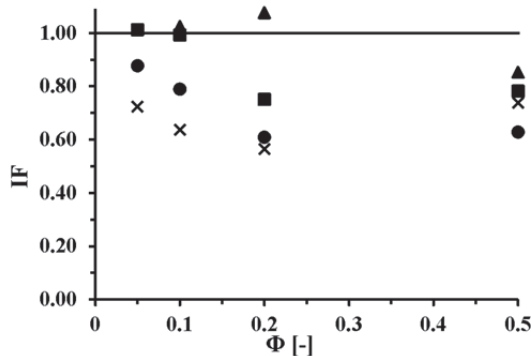


Figure 4.6 Integration factor based on net oxygen evolution rates versus duty cycle for four selected frequencies, x f = 5 Hz, ● f = 10 Hz, ■ f = 50 Hz, ▲ f = 100 Hz.

In this study oxygen yield on absorbed photons was determined (Figure 4.7), which so far has not been reported for flashing light experiments in a biological oxygen monitor.

The dependency of oxygen yield on duty cycle is shown in Figure 4.7. The highest yield for each flash frequency was found at a duty cycle of 0.1, which relates to a time-averaged PFD of about $115 \mu\text{mol m}^{-2} \text{s}^{-1}$. A lower duty cycle resulted in a decrease in yield which is similar to the observation in continuous light with PFDs below

$100 \mu\text{mol m}^{-2} \text{s}^{-1}$. In contrast to continuous light where a high yield was also observed for a PFD of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, a duty cycle of 0.2 (i.e time-averaged PFD of $230 \mu\text{mol m}^{-2} \text{s}^{-1}$) resulted in a decrease in yield except for the frequency of 100 Hz. The yield depended on the duty cycle but also on the flash frequency: the higher the flash frequency the higher the yield. The lowest yields were determined for the highest duty cycle, where the flash frequency did not have an influence anymore.

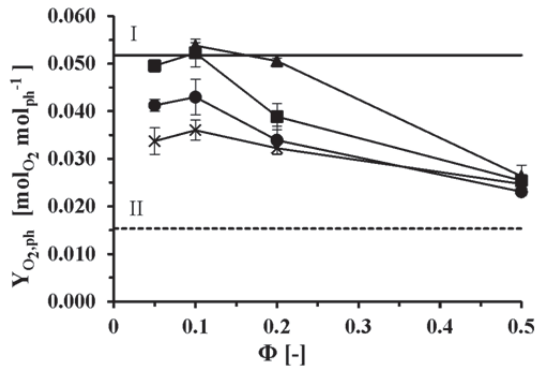


Figure 4.7 Oxygen yield on absorbed light energy versus duty cycle for four selected frequencies, x $f = 5$ Hz, ● $f = 10$ Hz, ■ $f = 50$ Hz, ▲ $f = 100$ Hz, I maximal yield measured under continuous illumination, II yield measured at $1079 \mu\text{mol m}^{-2} \text{s}^{-1}$, which is maximal yield expected for growth integration.

Discussion

The maintenance respiration rate (R_{ms}) in continuous light was $0.11 \mu\text{mol O}_2 \text{s}^{-1} \text{g}^{-1}$ as determined by the linear correlation between light respiration and net oxygen production rate. Each oxygen evolution measurement was started with a dark acclimation period of 5 min followed by an initial maintenance respiration measurement of 10 min. The initial maintenance respiration measurement of each experiment resulted on average in $0.10 \pm 0.02 \mu\text{mol O}_2 \text{s}^{-1} \text{g}^{-1}$, which is the same as derived from the data fit of equation (4.2). The initial maintenance respiration was measured at low dissolved oxygen concentrations: typically 15 % to 30 % air-saturation. If the maintenance respiration of dark acclimated algae was measured at a higher initial dissolved oxygen concentrations then it resulted in $0.25 \pm 0.03 \mu\text{mol O}_2 \text{s}^{-1} \text{g}^{-1}$ at 100 % air-saturation. This result was independent from the time of measurement: initial maintenance respiration, or maintenance respiration after exposure to different PFDs inside the BOM, but only dependent on dissolved oxygen concentration. In other words, there is a dependency of maintenance respiration on dissolved oxygen concentration.

Maintenance respiration is a part of respiratory activity during photosynthesis in the light. If this respiration is dependent on dissolved oxygen concentration then it could

add to the uncertainty of the oxygen evolution measurement. However, this uncertainty is not controllable because dissolved oxygen cannot be kept constant during the measurement, and, therefore, each point of interest was measured at least as a triplicate.

The oxygen yield on absorbed light energy in continuous light is influenced by maintenance respiration. At a photon flux density (PFD) below $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ the yield decreased due to a relative increase in maintenance (absolute maintenance is constant) to support cell metabolism. The highest net oxygen yield in continuous light was observed between a PFD of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. This range of PFDs is surprising because photosynthesis starts to become saturated already at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$, which was confirmed by a decrease in gross yield at a PFD of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. As a result, the maximal yield would have been expected around $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ where oxygen production rate still increases linear with PFD. Therefore, the influence of maintenance-associated respiration should not be underestimated at PFDs below $200 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The flashing light experiments show that full light integration based on net oxygen production rate is only possible at high frequencies ($> 50 \text{ Hz}$) and there is a strong dependence on the duty cycle which determines the time-averaged PFD. The duty cycles of 0.05 and 0.1 are favorable in terms of light integration also with low flash frequencies, whereas at a duty cycle of 0.5 the flash frequency has almost no influence on the absolute amount of light integration. In summary, presented data show that for full light integration the light flash should not be longer than 2 ms with a sufficient dark time not exceeding 20 ms.

Similar to the oxygen production rate the oxygen yield was always higher than the yield expected for growth integration (i.e. yield at $1079 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous light), and the yield under flashing light did not exceed the maximal yield measured under continuous light.

Data from this study show that light integration based on photosynthetic rate should not be the only criteria to evaluate the effect of flashing light. Based on the integration factor it could be concluded that the duty cycle of 0.05 and 0.5 showed the highest level of light integration. But, from the yield data it can be concluded that highest yields on light can be expected for a duty cycle of 0.1. In addition to the dependency of the yield on the duty cycle our data also showed that for duty cycles below or equal to 0.2 the yield decreased with decrease in flash frequency. In summary, the duty cycle should be

adjusted that the time-averaged PFD is photosynthetic rate limiting to achieve the highest yields.

Both oxygen production rate and yield data in flashing light show a similar trend than continuous illumination with the time-averaged PFD: If the incident PFD (continuous light) or time-average PFD (flashing light) is sub-saturating then highest yields can be observed in continuous and in flashing light. But, higher oxygen production rates will be observed with higher duty cycles, whereas yields will decrease. If the PFD is too low ($< 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) then the yield will also decrease because a relatively large fraction of light is needed to fulfill maintenance requirements. Therefore, the application of proper L/D cycles (and duty cycles) can lead to an increase in light conversion efficiency even at flash frequencies of 5 Hz and 10 Hz, which can be found in current PBR designs (Luo et al. 2003; Perner-Nochta and Posten, 2007; Pruvost et al. 2006).

The presented experiments represent a simplification of L/D cycles in photobioreactors. It was assumed that the incident PFD rather than local PFDs along the gradient or spatial average PFD of a gradient determine photosynthetic rate and oxygen yield (or biomass yield) on absorbed light energy. Under light attenuation conditions (i.e. high biomass density), which are relevant for commercial PBR operation, the light gradient, especially under over-saturating incident PFD, might lead to an increase in yield due to reduced exposure time to the incident over-saturating PFD. Thus, less improvement in both rate and yield might be obtained by the application of L/D cycles. Nevertheless, this simplification is an important step to understand the fundamental influence of flashing light and to improve mathematical models which can describe this influence. These models should include a mechanistic approach to be applicable for a wide range of L/D cycles and different incident PFDs. Based on these models it will be possible to find the optimal configuration of duty cycle and flash frequency to optimize yield and photosynthetic rate for a given PBR system and location.

Acknowledgements

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Chapter 5

The role of an electron pool during light/dark cycles in *Chlamydomonas reinhardtii*

Abstract

As a result of mixing and light attenuation in a photobioreactor (PBR), microalgae experience light/dark (L/D) cycles that can enhance PBR efficiency. One key element to optimize PBR efficiency is to be able to predict microalgal growth and productivity depending on environmental conditions, out of which light availability is the most important one.

The objective of this study was to develop and validate a mechanistic model that describes net photosynthetic oxygen evolution under flashing light based on the biomass specific light absorption rate and the dissipation rate of excess absorbed light. Net oxygen evolution is a direct measure of biomass growth.

The model describes photosynthetic oxygen evolution based on the availability of reducing equivalents (electrons), which result from the light reactions. Electrons are accumulated during the flash and serve as a pool for carbon dioxide fixation during the dark leading to partial or full light integration. Both, electron consumption and energy dissipation rates are based on a Monod-type kinetic.

The underlying assumption of an electron pool seems correct and its filling and emptying is depending on the flash time. The simulations show that if the dark time between flashes is not sufficiently long the pool will not be completely empty, and is responsible for a high energy dissipation rate. The measured oxygen production rates are described well, but the description of the energy dissipation rate will need further investigation.

For this chapter a manuscript for publication is in preparation.

Introduction

High photobioreactor (PBR) productivity is crucial to microalgae feed stock production and depends, amongst others, on a high biomass yield on light energy. One key element to design PBRs and optimize their productivity is to be able to predict microalgal growth and productivity depending on environmental conditions such as temperature, pH, nutrient availability and most importantly light availability.

If a PBR is exposed to (sun) light then light is attenuated along the PBR depth as a function of algae concentration and pigmentation. A high biomass concentration results in an illuminated zone with net photosynthesis and a dark zone with possible respiration. As a result of mixing algae experience flashing light; this is also called light/dark (L/D) cycles. These mixing-induced L/D cycles are suggested to lead to enhanced PBR efficiency (Degen et al., 2001; Hu and Richmond, 1996; Richmond, 1996).

Photosynthetic efficiency under a fast flashing light regime (frequency > 1 Hz) can be described by partial or full light integration (Phillips and Myers, 1954; Terry, 1986; Vejrazka et al., 2011). Under full light integration algae do not respond to the actual photon flux density (PFD) during the flash of an L/D cycle, but apparently to the lower, time-averaged PFD of the whole cycle (flash + dark time). If algae are grown under light limiting conditions then photosynthetic efficiency is high (Tredici, 2009; Vejrazka et al., 2012, manuscript submitted) and, therefore, full light integration results in a high photosynthetic efficiency.

To predict photosynthetic efficiency and PBR productivity models must become available, which describe the photosynthetic rate (oxygen evolution, specific growth rate) under dynamic light regimes.

One widely used model to describe photosynthetic oxygen evolution as a function of irradiance is the photosynthesis-irradiance relationship, or P-I curve (Jassby and Platt, 1976). This empirical relationship can describe oxygen evolution in a static light regime, thus it cannot predict algae growth or photosynthetic oxygen production under L/D cycles.

A first model aiming to describe the dynamics of photosynthesis under L/D cycles was developed by Baumert (1976). This model was based on the assumption that absorbed photons are reversibly changed into an intermediate form, which is a substrate for oxygen catalysis. The intermediate has a relaxation time constant, consequently, oxygen production proceeds partly during the dark and (partial) light integration could be

explained. Vejrazka et al. (2011) presented a hypothesis that algae have a pool of reducing equivalents available that can aid growth for short dark periods. This pool could be seen as the intermediate stage that has been described in Baumert's (1976) model and, therefore, be linked to intracellular metabolites.

More complex and dynamic models to describe photosynthesis are often based on the 3-state model originally developed by Eilers and Peeters (1988). This model describes photosynthesis based on a photosynthetic unit (PSU), which produces oxygen depending on the incident PFD. The PSU has three states: resting (functional), active (functional) or inhibited (non-functional). In this way photoinhibition due to over-saturating photon flux densities could be described as well.

The model of Eilers and Peeters (1988) was further developed by Rubio et al. (2003) and García-Camacho et al. (2012). The novelty of the model presented by Rubio et al. (2003) was that energy consumption was based on a metabolic control by an enzyme mediated process (Monod-type kinetic) and it included photoacclimation. The description of photoacclimation was further improved by García-Camacho et al. (2012). In contrast to Eilers and Peeters (1988) photoinhibition was described as a square root dependency of the incident PFD (Rubio et al., 2003) or as a power dependency (García-Camacho et al., 2012). In case of flashing light photoinhibition depended on the time-averaged PFD (Rubio et al., 2003), but light absorption was based on the incident PFD. One result of the model of Rubio et al. (2003) was that the activated PSUs could be used to prolong photosynthesis into short dark periods (sub-second L/D cycles). But, the extended model of García-Camacho et al. (2012) was not tested for sub-second L/D cycles

Photoinhibition or energy dissipation most likely depends not only on the average PFD (duty cycle) but also on the overall length of the flash and dark period, which was supported by data in Vejrazka et al. (2012, manuscript submitted). Data in Vejrazka et al. (2012, manuscript submitted) clearly showed that photosynthetic efficiency and oxygen production is dependent on both duty cycle and flash frequency.

The objective of the current study was to develop and validate a mechanistic model which describes photosynthetic oxygen evolution under flashing light based on the biomass specific light absorption rate and the dissipation rate of excess absorbed light energy.

The novelty of this dynamic model is that it also describes the energy dissipation rate based on the reduction state of the photosystem, which is expressed as a time-dependent amount of reducing equivalents (electrons). The simulated energy dissipation rate is

compared to values based on measurements. Furthermore, the model describes photosynthetic oxygen evolution based on the availability of electrons, which are accumulated during the flash and serve as a pool for photosynthesis during the dark.

Materials and Methods

Organism and medium

Chlamydomonas reinhardtii CC-124 wild type mt- 137c was obtained from the Chlamydomonas culture collection at Duke University (now Chlamydomonas resource center). The microalgae were cultivated in a Sueka high salt (HS) based liquid medium, composed of (amounts in g L⁻¹): NH₄Cl, 1.450; KH₂PO₄, 0.720; K₂HPO₄, 1.440; MgSO₄·7H₂O, 0.280; CaCl₂·2H₂O, 0.057; Na₂EDTA·2H₂O, 0.067 and 10 mL L⁻¹ of a 100 times concentrated Hutner's trace elements solution (Hutner et al., 1950). Axenic cultures were maintained in shake flasks placed on an orbital shake incubator at pH 7, 25 °C, air enriched with 2.5 %_{v/v} CO₂, and 60 – 80 μmol m⁻² s⁻¹ continuous illumination for at least one week before use as photobioreactor (PBR) inoculum.

Experimental procedure

Algae were pre-cultivated in a turbidostat operated photobioreactor (PBR) and acclimated to a sub-saturating photon flux density of 100 μmol m⁻² s⁻¹. The PBR operation procedure and dry weight measurement are described in (Vejrazka et al. 2012, manuscript submitted).

Oxygen evolution under different flashing light regimes was measured in a custom made biological oxygen monitor (BOM) as described in Vejrazka et al. (2012, manuscript submitted).

The flashing light regime was defined by the length of the square-wave flash (t_l , ms), dark time (t_d , ms), cycle time ($t_c = t_l + t_d$, ms), duty cycle ($\phi = t_l/t_c$, -), and flash frequency ($f = 1000/t_c$, Hz). The flash photon flux density (PDF_L , μmol m⁻² s⁻¹) was provided by two sides and set to a total of 1079 μmol m⁻² s⁻¹ (LED1: 536 μmol m⁻² s⁻¹, LED2: 543 μmol m⁻² s⁻¹). The photon flux density in flashing light was measured as described in Vejrazka et al. (2012, manuscript submitted).

Four different sets of flashing light experiments (Table 5.1) were measured: fixed dark time (9 ms (Set1), 18 ms (Set2), variable flash time) and fixed flash time (1 ms (Set3), 10 ms (Set4), variable dark time). The experiments were designed as such to get first insight in the dependency of energy dissipation on dark (recovery) time if a fixed amount

of energy is provided (fixed flash time). Second, to investigate the dependency of energy dissipation if a fixed dark time but varying energy input (variable flash time) is provided. The combination of flash times and dark times was chosen that a broad range of duty cycles and flash frequencies were covered.

Table 5.1 Overview of L/D cycle settings tested.

Set1				Set2				Set3				Set4			
t_f	t_d	f	Φ	t_f	t_d	f	Φ	t_f	t_d	f	Φ	t_f	t_d	f	Φ
[ms]	[ms]	[Hz]	-	[ms]	[ms]	[Hz]	-	[ms]	[ms]	[Hz]	-	[ms]	[ms]	[Hz]	-
1	9	100	0.100	1	18	53	0.053	1	1	500	0.500	10	5	67	0.667
2	9	91	0.182	2	18	50	0.100	1	2	333	0.333	10	10	50	0.500
3	9	83	0.250	3	18	48	0.143	1	3	250	0.250	10	20	33	0.333
4	9	77	0.308	4	18	45	0.182	1	4	200	0.200	10	30	25	0.250
5	9	71	0.357	5	18	43	0.217	1	5	167	0.167	10	40	20	0.200
6	9	67	0.400	6	18	42	0.250	1	9	100	0.100	10	50	17	0.167
9	9	56	0.500	8	18	38	0.308	1	12	77	0.077	10	70	13	0.125
18	9	37	0.667	12	18	33	0.400	1	18	53	0.053	10	90	10	0.100
36	9	22	0.800	18	18	28	0.500	1	19	50	0.050	10	120	8	0.077
								1	24	40	0.040	10	190	5	0.050
								1	39	25	0.025	10	240	4	0.040

Oxygen yield on absorbed light energy

The time-average biomass specific photon absorption rate ($q_{ph,abs}^{L/D}$, $\mu mol_{ph} g_{bm}^{-1} s^{-1}$) was determined based on the measured time-averaged PFD ($PF_{L/D}$, $\mu mol m^{-2} s^{-1}$, for protocol see Vejrazka et al., 2012, manuscript submitted) and the correlation between absorption rate and incident PFD under continuous light (see Vejrazka et al., 2012, manuscript submitted).

The net (n) oxygen yield ($Y_{O_2/ph,n}$, $mol_{O_2} mol_{ph}^{-1}$) or gross (g) oxygen yield ($Y_{O_2/ph,g}$, $mol_{O_2} mol_{ph}^{-1}$) on absorbed photons is the quotient of the measured net (n) oxygen production rate ($q_{O_2,n}$, $\mu mol_{O_2} g_{bm}^{-1} s^{-1}$) or gross (g) oxygen production rate ($q_{O_2,g}$, $\mu mol_{O_2} g_{bm}^{-1} s^{-1}$) and biomass specific photon absorption rate. The measured net oxygen evolution rate was converted into the gross oxygen evolution rate using the linear dependency of respiration rate on net oxygen evolution as described in Vejrazka et al. (2012, manuscript submitted).

$$Y_{O_2/ph,z} = \frac{q_{O_2,z}^{L/D}}{q_{ph,abs}}, z \in [n, g] \quad (5.1)$$

Photon dissipation rate

The biomass specific photon dissipation rate ($q_{ph,diss}$, $\mu mol_{ph} g_{bm}^{-1} ms^{-1}$) during the light flash was calculated according to equation (5.2). The maximal gross oxygen yield on

photons ($Y_{O_2,ph,g}^{\max}$) was calculated from data measured under continuous light as shown in Vejrazka et al. (2012, manuscript submitted). This maximal gross yield was the average ($0.085 \pm 0.020 \text{ mol}_{O_2} \text{ mol}_{ph}^{-1}$) of the gross yield of the three lowest PFDs (10, 30 and $42 \mu\text{mol m}^{-1} \text{ s}^{-1}$) measured. Photons are only absorbed during the light flash, therefore the time-averaged photon absorption rate ($q_{ph,abs}^{L/D}$) was divided by the duty cycle (ϕ) to calculate the flash specific absorption rate ($q_{ph,abs}, \mu\text{mol}_{ph} \text{ g}_{bm}^{-1} \text{ ms}^{-1}_{flash}$)

$$q_{ph,diss} = q_{ph,abs} \cdot \left(1 - \frac{Y_{O_2,ph,g}}{Y_{O_2,ph,g}^{\max}} \right) \quad (5.2)$$

The photon dissipation rate per millisecond of flash time (equation (5.2)) represents an average dissipation rate over the whole flash, thus not accounting for possible dynamics during the flash.

Model theory

The model is based on observations of specific oxygen production rate in flashing light. Partial or full light integration can only be achieved if photosynthesis, carbon dioxide reduction that leads to biomass growth, continues during the dark time of an L/D cycle. Therefore, algae must be able to store reducing equivalents or (i.e. electrons) liberated from water in a pool which can be used in the dark (Vejrazka et al., 2011). The general idea of this model (see scheme Figure 5.1) is to describe oxygen evolution and, as such, carbon dioxide reduction and growth, based on the availability of electrons, which are generated and consumed during the light flash. The excess electrons generated during the flash accumulate in a pool of reducing equivalents and are used during the following dark period. The waste product of the photosynthetic reaction is oxygen and its production is a measure of growth. Therefore, the oxygen production rate in the proposed model is coupled to the production of electrons (reducing equivalents).

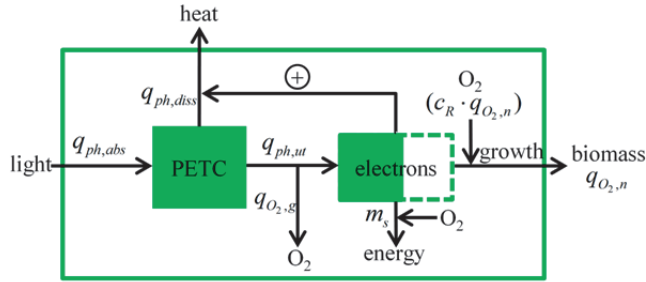


Figure 5.1 Flow chart of the proposed model, PETC – photosynthetic electron transport chain. + indicates a positive feedback regulation which is described as a Monod-type kinetic, box of electrons describes the pool that is filled during the flash and used during the dark, description of the rates can be found in the text.

Based on the incident PFD (i.e. flash intensity, $PF D_L, \mu mol m^{-2} ms^{-1}_{flash}$) and the average absorption cross section ($\bar{a}_{dw}^*, m^2 g_{bm}^{-1}$) a biomass specific photon absorption rate ($q_{ph,abs}, \mu mol_{ph} g_{bm}^{-1} ms^{-1}_{flash}$) can be calculated according to equation (5.3):

$$q_{ph,abs} = PF D_L \cdot \bar{a}_{dw}^* \quad (5.3)$$

If the photon absorption rate exceeds the maximal biomass specific photon utilization rate ($q_{ph,ut}, \mu mol_{ph} g_{bm}^{-1} ms^{-1}_{flash}$) then the difference will be dissipated as heat:

$$q_{ph,abs} = q_{ph,ut} + q_{ph,diss} \quad (5.4)$$

The biomass specific photon dissipation rate ($q_{ph,diss}, \mu mol_{ph} g_{bm}^{-1} ms^{-1}_{flash}$) depends on the length of the light flash but also the time available in between the flashes for the algae to utilize the reducing equivalents generated. In other words, it can be coupled to the redox state of the photosystem, which will be expressed as a concentration of electrons ($c_{e^-}, \mu mol_{e^-} g_{bm}^{-1}$). The feedback of the electron concentration on energy dissipation rate (Figure 5.1) is described as an arbitrarily chosen Monod-type kinetic:

$$q_{ph,diss} = \frac{q_{ph,diss}^{max} \cdot c_{e^-}}{K_s^{diss} + c_{e^-}} \quad (5.5)$$

A general mass balance for reducing equivalents, which are expressed as electrons ($c_{e^-}, \mu mol_{e^-} g_{bm}^{-1}$), is shown in equation (5.6) with:

- Volume of BOM ($V_{BOM} = 0.0122 L$)
- Biomass concentration in BOM ($c_x, g_{bm} L^{-1}$)
- Electron consumption rate ($r_{e^-}, \mu mol_{e^-} ms^{-1}$)

- gross yield of electrons on utilized photons ($Y_{e^-/ph,g}^{\max}$, $mol_{e^-} \cdot mol_{ph}^{-1}$)

$$\frac{d(V_{BOM} c_x c_{e^-})}{dt} = -r_{e^-} + (r_{ph,abs} - r_{ph,diss}) \cdot Y_{e^-/ph,g}^{\max} \quad (5.6)$$

The biomass concentration and volume of the BOM were constant during the oxygen evolution measurement and equation (5.6) can be simplified to equation (5.7). By dividing the overall rate (r) by the total biomass concentration in the BOM a biomass specific rate (q) is obtained.

$$\frac{dc_{e^-}}{dt} = -q_{e^-} + (q_{ph,abs} - q_{ph,diss}) \cdot Y_{e^-/ph,g}^{\max} \quad (5.7)$$

The electron consumption rate (q_{e^-} , $\mu mol_{e^-} \cdot g_{bm}^{-1} \cdot ms^{-1}$) is assumed to follow a Monod type kinetic (equation (5.8)) and is therefore directly coupled to cell metabolism as suggested by Rubio et al. (2003).

$$q_{e^-} = \frac{q_{e^-}^{\max} \cdot c_{e^-}}{K_s^{e^-} + c_{e^-}} \quad (5.8)$$

Assuming that four electrons are necessary to form one oxygen molecule the biomass specific gross oxygen production rate ($q_{O_2,g}$, $\mu mol_{O_2} \cdot g_{bm}^{-1} \cdot s^{-1}$) is calculated based on equation (5.9) with a conversion factor to convert from milliseconds to seconds. Equation (5.9) can be seen as a general Herbert-Pirt equation without maintenance and product formation because oxygen consumption for maintenance and light dependent respiratory activity follows in a later step.

$$q_{O_2,g} = q_{e^-} \cdot Y_{O_2/e^-} \cdot 1000, Y_{O_2/e^-} = 0.25 \quad (5.9)$$

The gross oxygen production rate reflects the rate of carbon dioxide reduction to triose sugars, which are substrate for real biomass growth. Part of the triose formed is respired again to provide additional energy (ATP) to support the growth reactions and to fulfill maintenance requirements. Therefore, the net oxygen production rate ($q_{O_2,n}$, $\mu mol_{O_2} \cdot g_{bm}^{-1} \cdot s^{-1}$, equation (5.10)) depends on respiratory oxygen consumption related to maintenance (m_s , $\mu mol_{e^-} \cdot g_{bm}^{-1} \cdot s^{-1}$), and growth (c_R , -) as described in Vejrazka et al. (2012, manuscript submitted). This description of the net oxygen production rate also implies that once all reducing equivalents are consumed, oxygen production rate will be negative. Furthermore, oxygen production in the model happens together with electron consumption, while in reality oxygen was already produced once the electrons are liberated from water during the flash. Nevertheless, this model characteristic does not

have any influence on the predicted time-averaged (for one full L/D cycle) oxygen production rate.

$$q_{O_2,n} = (q_{O_2,g} - m_s) \cdot (1 + c_R)^{-1} \quad (5.10)$$

The net oxygen production rate in equation (5.10) is time dependent. To obtain the steady-state oxygen production rate as measured in the BOM the simulated time-dependent oxygen production rate was averaged over one L/D cycle (see section *Model input and parameter estimation*, equation (5.12)).

Results and discussion

Energy dissipation rate

The energy dissipation rate is an important variable in the presented model. It determines how much absorbed light energy leaves the photosystem unused, and consequently, how much energy can be used to fill up the electron pool. The dissipation rate measured for a duty cycle of 1 (continuous light at flash intensity) is the reference value for all presented experimental sets and was $0.094 \mu\text{mol}_{ph} \text{g}_{bm}^{-1} \text{ms}_{flash}^{-1}$.

In Figure 5.2 the energy dissipation rate is presented for the different sets of flashing light experiments. Figure 5.2a shows the dissipation rate depending on the dark time for two different, fixed flash times (1 ms and 10 ms). These data show that the dissipation rate decreases for both flash times with an increase in dark time, whereas the decrease is faster for the shorter flash time. In case of a flash time of 1 ms the dissipation rate reaches zero for dark times above 24 ms, indicating first that these dark times are long enough for photosystem re-oxidation, and second that the following short flash does not over-saturate the photosystem. Therefore photosynthesis operates at maximal efficiency. In case of 10 ms flash time the dissipation rate reaches a stable level of about half the reference dissipation rate with increase in dark time. The results suggest that already at a flash time of 10 ms the flash is too long and the photosystem has insufficient time during the dark to completely recover from the flash, which leads to a higher energy dissipation. In other words, the photosystem might be partly inhibited.

In Figure 5.2b the energy dissipation rate is presented depending on the flash time for two different fixed dark times (9 ms and 18 ms). In both cases the dissipation rate increases with increase in flash time, whereas for the shorter dark time the initial (short flash time) dissipation rate is higher in comparison to the longer dark time. This result is in accordance with the other two sets of experiments which showed that an increase in dark time between the flashes leads to a decrease in energy dissipation.

Overall, these results show that the dissipation rate depends on the length of the flash or the total energy input into the photosystem but also the time the photosystem has in-between the flashes to recover or to re-oxidize.

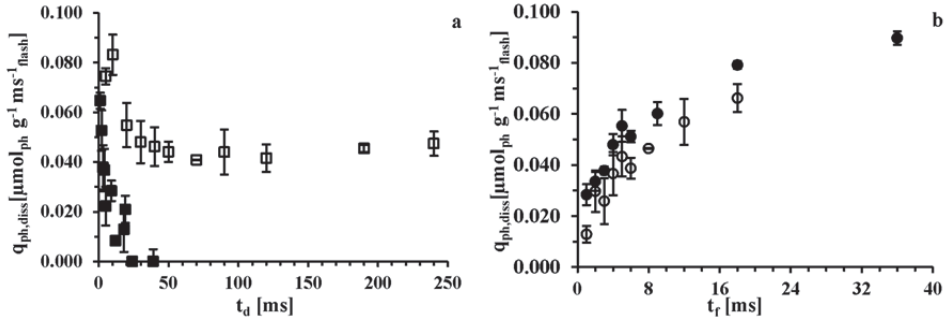


Figure 5.2: Photon dissipation rate versus a) dark time, b) flash time; a) ■ – constant flash time of 1 ms, variable dark time, □ - constant flash time of 10 ms, variable dark time; b) ● – constant dark time of 9 ms, variable flash time, ○ - constant dark time of 18 ms, variable flash time

Model input and parameter estimation

The model inputs are the flash photon flux density (PFD_L), specific absorption coefficient of the algae, the flash time and the dark time. However, the mathematical description of the biomass specific photon absorption rate (equation (5.3)) does not completely reflect the true absorption of light energy because a small light gradient exists in the BOM, which is complex to describe mathematically (e.g. Cornet et al., 1992). Because the presented model does not aim to describe light absorption by algae, the photon absorption rate for the simulation was based on the measured values ($q_{ph,abs}$, see section *Photon dissipation rate*).

The maximal photon dissipation rate ($q_{ph,diss}^{max}$, Table 5.2) equals the photon absorption rate: if the gross electron yield on photons (or gross oxygen yield) equals zero then no light energy is used and all absorbed energy must be dissipated as heat. During the experiments the flash intensity varied within a small range ($1090-1350 \mu\text{mol m}^{-2} \text{s}^{-1}$) due to different heating behavior of the LEDs under different flashing light regimes (see Vejrazka et al., 2012 manuscript submitted, for further explanations). Therefore, the maximal dissipation rate was almost constant and for the simulations the measured photon absorption rate was set as the maximal dissipation rate.

The maximal electron consumption rate (q_e^{max} , Table 5.2) is based on the maximal gross oxygen production rate observed under continuous light (Vejrazka et al. 2012, manuscript submitted). In order to calculate the maximal electron consumption rate it

was assumed that for the production of one molecule of oxygen four electrons are released.

The maintenance rate of oxygen consumption (m_s , Table 5.2) and the correlation factor (c_R , Table 5.2) between net photosynthesis and light respiration was obtained from P-I curve measurements (Vejrazka et al., 2012, manuscript submitted).

The maximal gross yield of electrons on photons ($Y_{e^-/ph,g}^{\max}$, Table 5.2) was obtained by multiplying the gross yield of oxygen on photons with four, assuming again that for the production of one molecule of oxygen four electrons are released

Table 5.2 Overview fixed parameter settings

parameter	value	unit
$q_{ph,diss}^{\max}$	$q_{ph,abs}$	$\mu mol_{ph} g_{bm}^{-1} ms_{flash}^{-1}$
$q_{e^-}^{\max}$	0.0091	$\mu mol_{e^-} g_{bm}^{-1} ms^{-1}$
m_s	0.107	$\mu mol_{O_2} g_{bm}^{-1} s^{-1}$
c_R	0.162	–
$Y_{e^-/ph,g}^{\max}$	0.347	$mol_{e^-} mol_{ph}^{-1}$

The half saturation constant for energy dissipation ($K_s^{diss}, \mu mol_{ph} g_{bm}^{-1}$) and electron consumption ($K_s^{e^-}, \mu mol_{ph} g_{bm}^{-1}$) were fitting parameters and fitted to data set 1 and 2. The fitting and simulation was done with the Matlab (2011a) `fminsearch` function, which is based on the Nelder and Mead algorithm (Lagarias, et al. 1998). The minimizing function (equation (5.11)) was based on the least squares and the variance (σ^2) of the data. In total 400 L/D cycles (time step 0.1 ms) were simulated for each setting to assure that equilibrium between dissipation and energy consumption was reached. The fitted function values were averaged for the last 50 simulated L/D cycles, whereas net oxygen production rate was averaged over the whole L/D cycle and energy dissipation rate was averaged over the light part of the L/D cycle (equation (5.12)).

$$f_{\min} = \sum_i \left(\frac{(f_{i,measured} - f_{i,fit})^2}{\sigma_i^2} \right), \quad f \in [q_{O_2,n}, q_{ph,diss}] \quad (5.11)$$

$$q_{O_2,fit} = \frac{1}{t_c} \cdot \int_{t=0}^{t_c} q_{O_2,fit}(t) dt, \quad q_{ph,diss} = \frac{1}{t_l} \cdot \int_{t=0}^{t_l} q_{ph,diss}(t) dt \quad (5.12)$$

The result of the fitted data can be seen in Figure 5.3 (Set1, Set2). The resulting half saturation constant for energy dissipation was $0.1181 \mu mol_{e^-} g_{bm}^{-1}$ and the half saturation

constant for electron consumption was $1.73 \cdot 10^{-4} \mu\text{mol}_e \text{g}_{bm}^{-1}$. The minimum function value of the minimizing function resulted in 263, which indicates a good fitting result.

The oxygen production rate in Set 1 (Figure 5.3a) is underestimated for flash times from 6 ms to 18 ms. At these two specific measurement points the oxygen production under flashing light was greater than the maximal oxygen production under continuous light, which was surprising and so far there is no explanation for this observation. However, the maximal oxygen production rate which is defined in the model was based on the observation under continuous light, therefore the model does not predict rates higher than that, and as a result, the oxygen production for 6 ms and 18 ms flash time was underestimated.

In general the oxygen production rate (average of one duty cycle) and the energy dissipation rate are fitted well by the model for both sets of experimental data. For long flash times the oxygen production rate reaches its maximal value and the energy dissipation rate approaches the value as expected for a duty cycle of 1 with increase in flash time. These results show that the underlying assumptions of an electron pool that can deliver energy during the dark part of the L/D cycle can describe our observations well. Furthermore, the coupling of the energy dissipation rate to the reduction state of the photosystem (internal electron concentration) also reproduces the observed energy dissipation rates well. The half-saturation constant for electron consumption is low in comparison to the simulated electron concentrations shown in Figure 5.5, which suggests mainly a reaction of zero order.

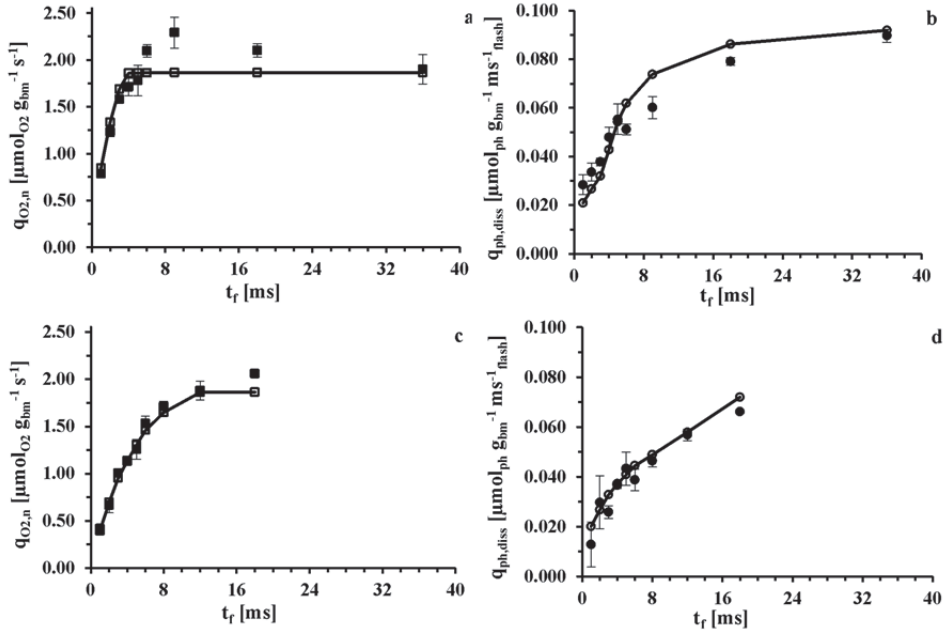


Figure 5.3: biomass specific net oxygen production rate versus flash time for a fixed dark time of a) 9 ms and c) 18 ms; biomass specific energy dissipation rate versus flash time for a fixed dark time of b) 9 ms and d) 18 ms; full symbols measured values, open symbols and line fitted values

Model validation

In order to show if the model is able to predict experimental data we performed two additional sets of experiments with fixed flash times but variable dark times (Set3, Set4). The results of the simulation and measurement data are shown in Figure 5.4. In general the model predicts the net oxygen production rates for both experimental sets well. Exceptions are very short flash times because the net oxygen production rate exceeded the maximal rate measured under continuous light (see *Model input and parameter estimation* for detailed explanation).

The energy dissipation rate for a flash time of 1 ms (Figure 5.4b) is predicted well for short dark times (< 8 ms). Afterwards it reaches a stable value for all following dark times while the real dissipation rate decreases to zero for a dark time of 24 ms and 40 ms. A similar effect is seen for the predicted dissipation rate in Set 4 (fixed flash time of 10 ms): initially the dissipation rate decreases with increase in dark time and reaches a stable level with further increase in dark time, which indicates a similar electron concentration during the light part for these L/D cycle settings. And, the predicted

dissipation rate is higher than the measured one, which is due to the half saturating constant for dissipation.

The simulations show that even if the net oxygen production rate is predicted well there are still some uncertainties in the prediction of energy dissipation. The energy dissipation rate is described as a Monod-type kinetic, which depends on the electron concentration. In order to establish oxygen production or growth a certain concentration of electrons is needed and the dissipation rate cannot be zero during light absorption.

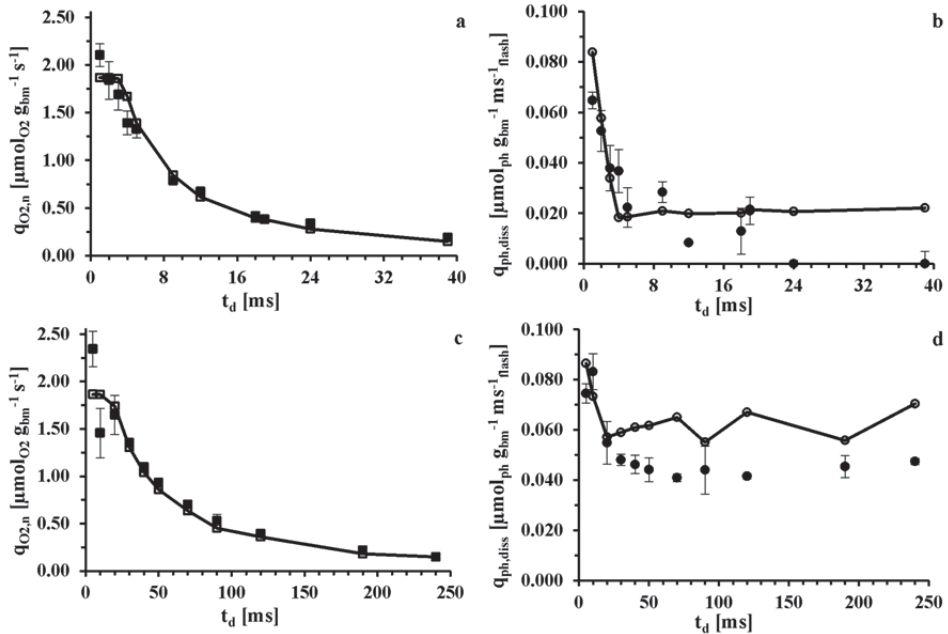


Figure 5.4: biomass specific net oxygen production rate versus dark time for a fixed flash time of a) 1 ms and c) 10 ms; biomass specific energy dissipation rate versus dark time for a fixed flash time of b) 1 ms and d) 10 ms; full symbols measured values, open symbols and line fitted values

An advantage of the present model in comparison to other dynamic models (e.g. Rubio et al. 2003) is that it predicts not only the net oxygen production rate but also the energy dissipation rate that can be experimentally determined if the relation between net oxygen production and overall respiration is known. However, the model of Rubio et al. (2003) also described measured oxygen production rates from other literature resources well.

The input parameter of this model is not the incident PFD but the measured photon absorption rate. With better knowledge and mathematical description of light absorption and scattering by algae it can be used as a basis to describe algae growth under dynamic

light conditions and thus improve photobioreactor productivity if the hydrodynamics of the system are known.

Furthermore, photoacclimation mechanisms might also be of some importance because they determine the optical cross section and as such the light absorption (see model for photoacclimation e.g. García-Camacho et al. (2012)). Probably photoacclimation will play the biggest role during the startup of a PBR. If a PBR is operated continuously at high cell densities (L/D cycle conditions) then algae will be acclimated to a low light regime and express a maximal optical cross section.

From a practical point of view, flash frequencies up to 25 Hz are the most interesting ones because measurements (Luo et al., 2003) and CFD simulations (Perner-Nochta and Posten, 2007; Pruvost et al., 2006) of fluid dynamics in current PBR designs resulted in L/D cycles of maximal 25 Hz. Data in from this study shows that the energy dissipation rate decreased with decrease in duty cycle (i.e. time-averaged PFD). The experimental set 4 contains most data points with practically relevant flash frequencies and in that range the dissipation rate is almost constant, thus the same amount of absorbed light energy is wasted and these L/D cycle combinations can be beneficial for PBR operation. However, the incident, time-averaged PFD should be in the optimal range of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ - $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ because otherwise net oxygen yield, and biomass yield, will decrease as shown in Vejrazka et al. (2012, manuscript submitted).

The experimental setup was a model setup, thus not a perfect reflection of real algae production PBRs. A light gradient during the flash will also have an influence on algae productivity (Takache et al., 2010; Brindley et al., 2011; Vejrazka et al. 2012), which needs validation with the proposed model. Nevertheless, this model is interesting for PBR design because it predicts oxygen production rates not only for the high frequencies but also for frequencies that are measured in PBRs (1 Hz to 25 Hz). It can be used as a basis for a growth model that simulates scenarios with different biomass concentrations and L/D cycles. Based on the predicted net oxygen production rates and growth stoichiometry of the algae (Vejrazka et al., 2011) the specific growth rate, expected biomass yields and volumetric productivities can be determined.

Simulated electron concentration during L/D cycles

In order to get a better insight in the dynamics of the model, Figure 5.5 shows the simulated electron concentration at the end of the light part of the L/D cycle (filled pool) and the electron concentration at the end of the dark part of the L/D cycle. In other

words, Figure 5.5 shows the range between which the electron concentration is oscillating.

The maximal electron concentration is increasing with increase in flash time for a fixed dark time (Figure 5.5a). The initial increase is similar for both dark times. However, at a flash time of about 5 ms the increase for the longer dark time (18 ms) is less than for the short dark time (9 ms), which is due to the fact that at longer dark times the pool is completely emptied in comparison to the short dark time. Only at flash times greater than 12 ms the pool is not completely emptied anymore in case of 18 ms dark time.

The maximal concentration of electrons seems to approach a maximum with increase in flash time. This maximum is due to the increase in energy dissipation rate, and consequently, a decrease in electron yield on absorbed photons. In comparison to Rubio et al. (2003) our model does not need a maximally defined concentration of photosynthetic units (or electrons), but it is inherent to the model dynamics itself.

Figure 5.5b shows the electron concentration for the fixed flash times and variable dark times. The electron concentration reached at the end of the light part is decreasing with increase in dark time and reaches a constant value. The high electron concentrations for short dark times are due to the fact that at the end of dark cycle the pool is not completely empty. But, if the pool is completely emptied then the electron concentration during the flash will always reach the same value for one fixed flash time because during the flash the same amount of energy is provided. This similar maximal electron concentration for a broad range of dark times also explains why the energy dissipation rate (Figure 5.4b, d) with its current description does not fit as good for longer dark times as it does for shorter dark times: the dynamics in electron formation are the same if after the dark period the pool is empty. As a consequence of these dynamics the dissipation rate will also be the same for L/D settings with the same flash time. The Monod-type dependency between energy dissipation and electron concentration is not the best description. Other factors like the light-dark ratio and overall cycle length might also affect factors triggering energy dissipation but are not completely represented by solely the electron concentration. Therefore, the description of the dissipation rate should be further studied and maybe adjusted by defining e.g. additional boundary conditions based on optimal L/D cycle settings without dissipation.

The concentration of electrons during the flash in combination with the low half saturation constant for electron indicates a zero order kinetic. Therefore, oxygen

production proceeds mostly at its maximal rate during the light part but also during the dark part as long as electrons are available.

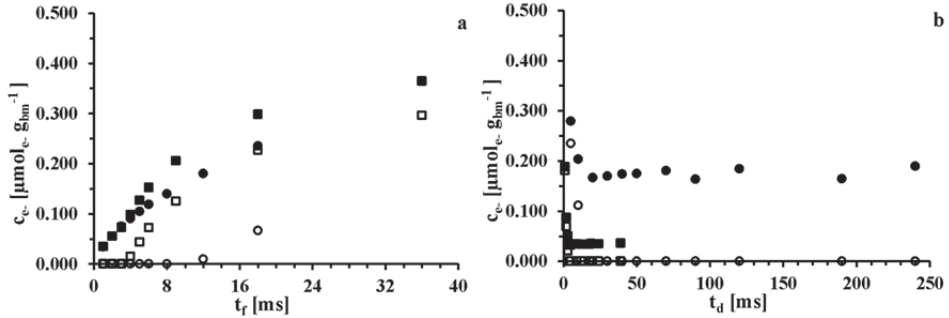


Figure 5.5: a) electron concentration versus flash time for ■/□ fixed dark time of 9 ms (Set1) and ●/○ fixed dark time of 18 ms (Set2), full symbols: max electron concentration at the end of the flash, open symbols electron concentration at the end of dark period, b) electron concentration versus flash time for ■/□ fixed flash time of 1 ms (Set3) and ●/○ fixed flash time of 10 ms (Set4), full symbols: maximal electron concentration at the end of the flash, open symbols electron concentration at the end of dark period

Even though the model includes energy dissipation, the underlying idea of an electron pool is a lumped assumption. First, the pool is probably not composed of a single chemical entity but several: e.g. NADPH, intermediates of the Calvin cycle. Second, during the dark time the electron transport chain can equilibrate again and, thus, work more efficiently once the next flash arrives, which is probably more important for longer dark times or very short flash times. Third, with increase in flash time the proton gradient across the thylakoid membrane could slowly build up and deliver additional ATP for C-fixation and reduce respiratory activity and loss of triose.

For *Chlamydomonas reinhardtii* a maximal NADPH concentration of $0.151 \mu\text{mol}_{\text{NADPH}} \text{g}_{\text{bm}}^{-1}$ was calculated based on literature data (see Appendix). One NADPH molecule is the carrier of two electrons, therefore, a maximal concentration of $0.302 \mu\text{mol}_{\text{e}^-} \text{g}_{\text{bm}}^{-1}$ based on NADPH can be available. The maximal simulated electron concentration was $0.351 \mu\text{mol}_{\text{e}^-} \text{g}_{\text{bm}}^{-1}$, thus above the maximal value based on literature data. These results suggest that NADPH could be a major contributor to the electron storage effect but it is not the only contributor.

In conclusion, the model predicts measured oxygen evolution rates and energy dissipation rates well for the experimental data sets described in this study but the description of the energy dissipation rate will need further investigation. The underlying assumption of an electron pool seems correct and its filling and emptying is depending on the flash time, and it is suggested that NADPH is most likely the major contributor to the electron storage effect.

Appendix

One molecule contributing to the electron storage effect is probably NADPH. Forti et al. (2003) reported a NADP + NADPH content for low light grown *Chlamydomonas reinhardtii* of 4.66 nmol mg⁻¹_{Chl}. In addition, Gans and Rebeille (1988) reported a total NADP + NADPH content of 11 nmol mg⁻¹_{Chl} for a different *Chlamydomonas reinhardtii* strain, which results in an average of 7.83 nmol mg⁻¹_{Chl} total NADP + NADPH for both studies.

Fortis et al. (2003) showed that the NADPH/NADP ratio depends on the incident photon flux density and reported a value of 1.5 for over-saturating light. Gans and Rebeille (1988) describe a maximal ratio of 2.5. For simplicity the ratio of these studies is averaged and results in 2. The initial (during dark) NADPH/NADP ratio in Fortis et al. (2003) was 1.55 and in Gans and Rebeille (1988) 1.25, which gives an average of 1.4. The difference in initial ratio and maximal reported ratio is 0.6, which can be used as electron storage. As a conclusion 2.94 nmol_{NADPH} mg⁻¹_{Chl} could be maximally available at the end of a light flash.

In Kliphuis et al. (2011) an average chlorophyll concentration of 51.4 mg_{Chl} g_{bm}⁻¹ was reported for low light acclimated *Chlamydomonas reinhardtii*. This chlorophyll concentration in combination with the reported NADPH content results in an average maximal NADPH concentration of 0.151 μmol_{NADPH} g_{bm}⁻¹.

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Chapter 6

Photosynthetic efficiency of *Chlamydomonas reinhardtii* in attenuated, flashing light

Abstract

As a result of mixing and light attenuation, algae in a photobioreactor (PBR) alternate between light and dark zones and, therefore, experience variations in photon flux density (PFD). These variations in PFD are called light/dark (L/D) cycles.

The objective of this study was to determine how these L/D cycles affect biomass yield on light energy in microalgae cultivation.

For our work we used controlled, short light path, laboratory, turbidostat-operated PBRs equipped with a LED light source for square-wave L/D cycles with frequencies from 1 Hz to 100 Hz. Biomass density was adjusted that the PFD leaving the PBR was equal to the compensation point of photosynthesis.

Algae were acclimated to a sub-saturating incident PFD of $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ for continuous light. Using a duty cycle of 0.5, we observed that L/D cycles of 1 Hz and 10 Hz resulted on average in a 10 % lower biomass yield, but L/D cycles of 100 Hz resulted on average in a 35 % higher biomass yield than the yield obtained in continuous light.

Our results show that interaction of L/D cycle frequency, culture density and incident PFD play a role in overall PBR productivity. Hence, appropriate L/D cycle setting by mixing strategy appears as a possible way to reduce the effect that dark zone exposure impinges on biomass yield in microalgae cultivation. The results may find application in optimization of outdoor PBR design to maximize biomass yields.

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Introduction

Renewable feed stocks for production of bulk chemicals or biofuels are only competitive with fossil based feed stocks if low production costs at large-scale can be achieved. Production costs of microalgal feed stocks depend, among others, on high photobioreactor (PBR) productivity (Norsker et al., 2011). For optimal design of outdoor, microalgae photobioreactors a high photosynthetic efficiency, which is biomass yield on light energy, is necessary to achieve high areal productivities.

A PBR is exposed to (sun) light at its surface and light is attenuated as a function of algae concentration. Light attenuation results in an exponential decrease in photon flux density (PFD) as a function of PBR depth. A higher biomass concentration results in a smaller illuminated zone with net photosynthesis, and a larger dark zone with respiration. As a result of mixing algae experience variations in PFD, which are light/dark (L/D) cycles. These mixing-induced L/D cycles can lead to enhanced PBR efficiency (Hu and Richmond, 1996; Richmond, 1996; Degen et al., 2001).

Algae growing at a saturating, incident PFD, or an over-saturating one, express a low biomass yield on light energy. If algae are grown under light limiting conditions then biomass yield will be high (Tredici, 2009). To evaluate the effect of a dynamic light regime on biomass yield, the theoretical concept of light integration is helpful (Phillips and Myers, 1954; Terry, 1986). Under ideal conditions of full light integration algae do not experience the actual PFD during the flash of an L/D cycle, but rather the lower time-averaged PFD of a whole cycle. In other words, biomass yield will be as high as under cultivation with continuous, limiting PFD. As a consequence of full light integration a high-density, well-mixed algae culture would grow more efficient especially at high (over-) saturating PFDs.

Algae response to different (dynamic) light regimes was studied by photosynthetic rate measurements based on oxygen evolution rate (Kok and Burlew, 1953; Matthijs et al., 1996; Nedbal et al., 1996; Terry, 1986) or specific growth rate (Phillips and Myers, 1954; Rosello-Sastre, 2010; Vejrazka et al., 2011; Xue et al., 2011). These photosynthetic rate measurements (oxygen evolution rate and growth rate) showed that sub-second L/D cycles are necessary to measure light integration.

L/D cycles were generally simulated by flashing a light source in a square-wave pattern on a diluted algae culture. In a diluted algae culture mutual shading is minimized and, therefore, light attenuation during the flash. As a consequence of minimal light attenuation all algae are exposed to a similar PFD. In practice, however, a high biomass

concentration is expected, which leads to full light attenuation along the depth of the PBR.

Brindley et al. (2011) compared oxygen production of *Scenedesmus almeriensis* under light attenuation and under minimized light attenuation for flash frequencies from 0.1 Hz to 50 Hz with (over-) saturating flash intensities. If the flash frequency was greater than 10 Hz then the oxygen production was greater under light attenuation but for flash frequencies below 10 Hz no difference was observed. In other words, L/D cycles might be more beneficial if light is fully attenuated by algae.

Consequently, further quantitative understanding of the effect of mixing-induced L/D cycles on biomass yield is necessary but studies under defined PBR conditions are, however, not available.

We want to show the potential impact of sub-second L/D cycles on biomass yield of PBRs that are operated under full light attenuation and its dependency on flash frequency. Light/dark cycle experiments are an analogy to a PBR operated with a dark zone, therefore data from these experiments can be used to estimate the effect of a dark zone on biomass yield.

We will discuss light integration under L/D cycles, which simulate a high biomass density, with flash frequencies from 1 Hz to 100 Hz in comparison to reference experiments under continuous illumination. We will outline, furthermore, the importance to use biomass yield on light energy to evaluate light integration and give an outlook on points of consideration for PBR operation.

Materials and Methods

Organism and medium

Chlamydomonas reinhardtii CC-124 wild type mt- 137c was kindly provided by Thomas Happe from Bochum University. The microalgae were cultivated in a Sueka high salt (HS) based liquid medium, composed of (amounts in g L⁻¹): NH₄Cl, 1.450; KH₂PO₄, 0.720; K₂HPO₄, 1.440; MgSO₄·7H₂O, 0.280; CaCl₂·2H₂O, 0.057; Na₂EDTA·2H₂O, 0.067 and 10 mL L⁻¹ of a 100 times concentrated Hutner's trace elements solution (Hutner et al., 1950). Axenic cultures were maintained in shake flasks placed on an orbital shake incubator at pH 7, 25 °C, air enriched with 2.5 %_{v/v} CO₂, and 60 – 80 μmol m⁻² s⁻¹ continuous illumination for at least one week before use as photobioreactor (PBR) inoculum. The PBR medium contained additionally 0.420 g L⁻¹ of NaHCO₃ to prevent carbon limitation.

Photobioreactor setup

The PBR setup is similar to the one described in Vejrazka et al. (2011). The PBR system (Photobioreactor FMT 150, Nedbal et al., 2008) was purchased from Photon System Instruments (PSI, Brno, Czech Republic) and equipped with a blue-red LED panel emitting light at 450 nm and 630 nm. The PBR was operated as a turbidostat (axenic); each experiment was done in a biological duplicate in two independent PBRs and started with a dry weight concentration of $\approx 0.05 \text{ g}_{\text{bm}} \text{ L}^{-1}$.

Temperature was regulated at 25 °C and culture was mixed by sparging air at a flow rate of $1 \text{ L}_{\text{gas}} \text{ L}_{\text{culture}}^{-1} \text{ min}^{-1}$. The PBR cuvette (length x width x height = 100 x 25 x 195 mm) was filled to 150 mm height resulting in an illuminated liquid working volume of 375 mL. The pH was kept constant at 7.0 via pulse-wise CO_2 addition to the air stream.

The outgoing photon flux density (PFD), which can be related to the turbidity, was measured at the rear side of the PBR cuvette with a Hamamatsu S1227-33BR silicon photodiode. The amplified signal was processed via an A/D converter (NI USB-6008, National Instruments™, Woerden, The Netherlands) and a LabView (National Instruments™, LabView 7.1) program. If the outgoing PFD fell below a set threshold then the culture was diluted with fresh medium.

The specific growth rate during steady state ($\mu(t)$, $\text{g}_{\text{bm}} \text{ g}_{\text{bm}}^{-1} \text{ h}^{-1}$) was calculated according to equation (6.1) (Vejrazka et al., 2011): PBR volume (V_R , L), biomass concentration in the PBR (c_x^R , $\text{g}_{\text{bm}} \text{ L}^{-1}$), biomass concentration in the overflow (c_x^{out} , $\text{g}_{\text{bm}} \text{ L}^{-1}$) at time of sampling (daily) and outgoing flow rate ($\dot{F}_{\text{out}}(t)$, L h^{-1}).

$$\mu(t) = \frac{\dot{F}_{\text{out}}(t)}{V_R} \cdot \frac{c_x^{\text{out}}}{c_x^R} \quad (6.1)$$

The specific growth rate was determined for 6 h intervals. The average of 6 h intervals for the last 72 h of steady state is shown. To assess if steady state was reached, biomass concentration, and optical density inside the PBR, and overflow had to stay constant for the preceding 72 h.

Light regime

The flashing light regime was defined by the length of the square-wave flash (t_l , ms), dark time (t_d , ms), cycle time ($t_c = t_l + t_d$, ms), duty cycle ($\phi = t_l/t_c$, -), and flash frequency ($f = 1000/t_c$, Hz). By dividing the incident, time-averaged photon flux density ($\text{PFD}_{0,L/D}$, $\mu\text{mol m}^{-2} \text{ s}^{-1}$) with the duty cycle (ϕ) the incident, flash photon flux density ($\text{PFD}_{0,L}$, $\mu\text{mol m}^{-2} \text{ s}^{-1}$) could be re-calculated.

Light regimes are summarized in Table 6.1. The incident photon flux density (PFD) for reference 1 (continuous illumination) and flashing light regimes was set to $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ but varied from 220 - $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 6.1). For reference 2 (continuous illumination) the incident, time-averaged PFD of one L/D cycle was used: $110 \mu\text{mol m}^{-2} \text{s}^{-1}$. In Table 6.1 measured values for the PFD are shown. During all experiments light was attenuated along the light path of PBR by controlling the outgoing PFD during the light period. This PFD was set to $10 \mu\text{mol m}^{-2} \text{s}^{-1}$, which is enough to allow net photosynthesis and should ensure maximal productivity of a PBR system (Takache et al., 2010)

Table 6.1 Overview of light regimes tested.

#	Experiment	$PFD_0 / PFD_{0,L}$	PFD_{out}	ϕ	f	t_f
		[$\mu\text{mol m}^{-2} \text{s}^{-1}$]		[-]	[Hz]	[ms]
1	reference 1	225	10	1.0	0	-
2		221	10	1.0	0	-
3	reference 2	110	11	1.0	0	-
4		108	10	1.0	0	-
5	100Hz	224	10	0.5	100	5
6		227	10	0.5	100	5
7	10Hz	236	9	0.5	10	50
8		252	12	0.5	10	50
9	1Hz	250	12	0.5	1	500
10		238	9	0.5	1	500

Each experiment was done in a biological duplicate in two independent PBRs. The PFDs are the measured values during continuous illumination or during the flash. PFD_0 – incident photon flux density continuous light, $PFD_{0,L}$ – incident photon flux density flash, PFD_{out} – photon flux density leaving the PBR, ϕ - duty cycle, f – flash frequency, t_f – flash length.

Light measurement

Under continuous illumination the incident PFD ($PFD_0, \mu\text{mol m}^{-2} \text{s}^{-1}$) was measured at the position of the light exposed surface of the PBR cuvette at 35 equally distributed points and averaged into PFD_0 . This was done with a LiCor 190-SA 2π PAR (400-700 nm) quantum sensor (LiCor, Lincoln, Nebraska) before and after each experiment.

The spectral distribution (400 – 700 nm) of the light source was measured at a reference position with a fiber optic CCD based spectroradiometer (AvaSpec-2048 detector, Fiber FC-IR100-1-ME, Avantes, Eerbeek, The Netherlands). The same spectroradiometer was used to measure the PFD during the flashing light. A detailed description of the light measurement can be found in the appendix.

Biomass determination

For dry weight measurement Whatman GF/F glass microfiber filters (\varnothing 55 μm , pore size = 0.7 μm) were dried (24 h, 95 $^{\circ}\text{C}$), cooled down to room temperature in a desiccator ($> 2\text{h}$) and weighed. The sample (< 5 mg dry weight) was filtrated with a constant vacuum (0.44 bar, absolute), washed three times with MilliQ water, dried (24 hours, 95 $^{\circ}\text{C}$), cooled down in a desiccator ($> 2\text{h}$) and weighed again. The weight difference between empty and algae containing filter was the dry weight. Each sample was measured in triplicate. The steady state biomass concentration is the average biomass concentration based on three measurements which were done in the last 72 h.

Dry weight specific absorption coefficient

Algae light absorption was measured in a specialized spectrophotometer setup to minimize the effect of light scattering on absorption measurement. A sample (≈ 15 mL) was centrifuged (15 min, 4500 rpm, 4 $^{\circ}\text{C}$) and the pellet was re-suspended in a defined part of the supernatant (on weight basis) to reach a final extinction at 680 nm (chlorophyll absorption peak) between 1.8 and 2.2, as measured in a 1 cm cuvette in a spectrophotometer (Beckman DU[®]640, Beckman Coulter, USA). The absorbance was measured with a fiber optic CCD based spectrophotometer (Avantes, Eerbeek, The Netherlands). The sample containing cuvette (Hellma, 100.099-OS, 2 mm light path) was illuminated with an AvaLight-Hal light source via a FC-IR600-1-M fiber, which was equipped with a collimating lens. An integrating sphere (AvaSphere-50) was directly placed behind the cuvette and connected to an Avantes Avaspec-2048 detector via a FC-IR600-1-M fiber. The resulting absorbance was measured from 400 nm to 750 nm ($\Delta\lambda = 1$ nm). The average absorbance from 740 nm to 750 nm was subtracted from the absorbance between 400 nm and 700 nm, thus correcting for residual scattering (Dubinsky et al., 1986). The wavelength-dependent absorption coefficient ($a_{dw}^*(\lambda)$, $\text{m}^2 \text{g}_{bm}^{-1}$, equation (6.2)) was calculated based on absorbance (ABS_{λ}) at wavelength λ , dry weight of the sample (c_x , $\text{g}_{bm} \text{m}^{-3}$), light path of the cuvette (l , m), and 2.3026 (conversion factor common logarithm to natural logarithm):

$$a_{dw}^*(\lambda) = \frac{ABS_{\lambda} \cdot 2.3026}{c_x \cdot l} \quad (6.2)$$

From $a_{dw}^*(\lambda)$ the average specific absorption coefficient (\bar{a}_{dw}^* , equation (6.3)) was calculated with 300 intervals in the measured range.

$$\bar{a}_{dw}^* = \frac{\sum_{\lambda=400nm}^{700nm} a_{dw}^*(\lambda)}{300} \quad (6.3)$$

Biomass yield on light energy

The light absorption rate inside the PBR ($r_{ph,tot}$, $mol_{ph} s^{-1}$ equation (6.4)) was calculated based on the incident PFD spectra under continuous light ($PFD_0(\lambda)$) or flashing light ($PFD_{0,L/D}(\lambda)$), absorbed light during experiment (i.e. based on light transmission $T(\lambda)$, see appendix and section light measurement), illuminated surface (A_{light} , m^2) and conversion to mol (1×10^{-6}):

$$r_{ph,tot} = A_{light} \cdot 1 \times 10^{-6} \cdot \sum_{\lambda=400nm}^{700nm} i \cdot \Delta\lambda \cdot (1 - T(\lambda)) \quad i = PFD_0(\lambda), PFD_{0,L/D}(\lambda) \quad (6.4)$$

The biomass yield ($Y_{x/ph}$, $g_{bm} mol_{ph}^{-1}$, equation (6.6)) is the quotient of PBR biomass production rate (r_x , $g_{bm} s^{-1}$, equation (6.5)) and the photon absorption rate ($r_{ph,tot}$, $mol_{ph} s^{-1}$).

$$r_x = \mu \cdot c_x^R \cdot V_R \quad (6.5)$$

$$Y_{x/ph} = r_x / r_{ph,tot} \quad (6.6)$$

Results and discussion

We show results for a set of turbidostat experiments under full light attenuation, as in algae production PBRs. The flash frequencies were set from 1 Hz to 100 Hz to consider that measurements (Luo et al., 2003) and CFD simulations (Perner-Nochta and Posten, 2007; Pruvost et al., 2006) of fluid dynamics in current PBR designs resulted in L/D cycles of maximal 25 Hz.

We chose a sub-saturating incident PFD because Cuaresma et al. (2011) showed that higher biomass yields can be achieved by diluting the incident PFD a PBR receives, which is placing PBRs vertically in each other's shade. In addition, Tredici (2010) showed that algae biomass productivity can vary between 80 and 120 $t ha^{-1} year^{-1}$ in regions like North America and middle and north of Europe. Incident PFDs in these regions are not always saturating in the first half of the morning or the second half of the afternoon.

Biomass yield on light energy

A limited number of studies on L/D cycles have been done in turbidostat operated PBRs and only one reported biomass yields (Vejrazka et al., 2011) for continuous, turbidostat experiments. Vejrazka et al. (2011) emphasized to use biomass yield to evaluate light

integration because the biomass yield also includes light absorption and not only biomass growth.

The biomass yield under $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous illumination (reference 1) was $0.61 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ and the biomass yield under $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous illumination (reference 2) was $0.79 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ (Figure 6.1).

Under flashing light the biomass yield reached full light integration at very short flashes of 100 Hz ($t_f = 5 \text{ ms}$). In other words, algae did not experience the PFD during the flash but the time-averaged PFD of one L/D cycle. Lower flash frequencies of 10 Hz and 1 Hz ($t_f = 50 \text{ ms}$, 500 ms) resulted on average in a 10 % lower biomass yield ($0.56 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$) as for reference 1 ($0.61 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$, no L/D cycles). As a comparison Janssen et al. (2000) reported a minimum decrease in biomass yield of 25 % for longer L/D cycles ($f \leq 0.17 \text{ Hz}$) with a duty cycle of 0.5.

At the lower flash frequencies (longer flash times) no light integration was observed, hence algae responded to the PFD during the flash, and, consequently, there is no positive effect of L/D cycles on biomass yield under light attenuation with a low incident PFD. But, the positive effect of the light dilution strategy was confirmed with the high biomass yield in experiment reference 1 (continuous light, $220 \mu\text{mol m}^{-2} \text{s}^{-1}$) in comparison to illumination with saturating PFDs (Takache et al., 2010; Vejrazka et al., 2011). And, in case of flashing light a high biomass yield was still achieved although no light integration occurred at longer flash times (50 ms – 10 Hz, and 500 ms – 1 Hz).

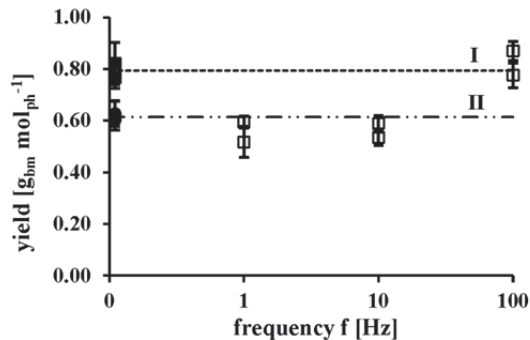


Figure 6.1 Biomass yield on light energy versus flash frequency. Continuous illumination: ■ (I) $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ (reference 2) ● (II) $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ (reference 1), flashing light: □. For experimental conditions see Table 1. Error bars represent the maximal standard deviation calculated based on the law of error propagation, which takes the standard deviation of the biomass concentration and the specific growth rate into account.

The range of photosynthetic enhancement, which can be defined as the difference in biomass yield between reference 1 and reference 2, was lower in comparison to an over-saturating incident PFD (Vejrazka et al., 2011; Xue et al., 2011). Under over-saturating incident PFDs biomass yield is low in comparison to light limiting incident PFDs and, as a consequence, L/D cycles will have a stronger effect on biomass yield in case of over-saturating incident PFDs.

Full light integration in terms of biomass yield on light energy was reached at a flash frequency of 100 Hz, which was the same in Vejrazka et al. (2011) but for minimized light attenuation. The flash time was, however, different: 5 ms in this study compared to 1 ms in Vejrazka et al. (2011). In both studies the incident, time-averaged photon flux density (PFD) was about $100 \mu\text{mol m}^{-2} \text{s}^{-1}$. This PFD is photosynthetic rate limiting for *C. reinhardtii*, a condition which seems to be required to achieve (full) light integration at flash frequencies of 100 Hz. We conclude that the optimal flash time to achieve full light integration is not a fixed value but a function of flash intensity and following dark period. The maximal dark time will be limited though; because at a certain point maintenance losses will increase (Kliphuis et al., 2011a) and undo biomass yield enhancement.

Biomass yields obtained in continuous light were lower than the ones measured in other studies under full light attenuation. Data in Takache et al. (2010) corresponds to a yield of $0.95 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ and $1.24 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ under $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous illumination. Kliphuis et al. (2011b) determined a maximal yield of $1.0 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$ under $635 \mu\text{mol m}^{-2} \text{s}^{-1}$ continuous illumination. The difference in yield could be attributed to different light sources used: white LEDs (Takache et al., 2010), and tungsten-halogen lamps (Kliphuis et al., 2011b). Both, white LEDs, and tungsten-halogen lamps also emit green light and the contribution of blue light is much lower than in our study. The emission of blue light in the white LED panel (Takache et al., 2010) was 32 % of total PAR photons and in the blue-red panel (this study) it was 62 % of total PAR photons. The tungsten-halogen lamps (Kliphuis et al., 2011b) emitted almost no blue light, which is strongly absorbed by algae (Figure 2a).

The lower efficiency of blue light was already demonstrated by quantum yield measurements of *Chlorella* (Emerson and Lewis, 1943) and is also known for higher plants (McCree, 1972). An additional explanation for the higher biomass yield in Takache et al. (2010) and Kliphuis et al. (2011b) in comparison to this study is the light absorption rate per amount of biomass. Algae have an absorption maximum (Figure 2a)

for blue light and, as a consequence, it is strongly absorbed in the first layers of the PBR. If green light is present in the spectrum of the lamps then it will penetrate deeper inside the PBR and light is more evenly distributed over algae present and they could grow more efficiently. A final conclusion about the influence of blue light, however, cannot be drawn yet because besides spectral differences between the different studies every PBR system was different with respect to light path and optics (beam angle and beam spread).

In this work 95 % of the light entering the PBR (based on a PFD leaving the PBR of $10 \mu\text{mol m}^{-2} \text{s}^{-1}$) was absorbed by the algae. This operation procedure should ensure maximal productivity of a PBR system (Takache et al., 2010) and is practically relevant. If higher incident PFDs are applied to a PBR system then more light will be absorbed if the outgoing PFD is fixed to $10 \mu\text{mol m}^{-2} \text{s}^{-1}$. An increase in biomass concentration in these scenarios will lead to a dark zone in the PBR. And L/D cycle experiments presented here can be interpreted as operating a PBR with a dark zone the same size as the illuminated zone ($\Phi=0.5$). Following Beer-Lambert's law such a dark zone would be introduced in a PBR by doubling the biomass concentration. As a consequence of the decreased biomass yield under L/D cycles the volumetric productivity will also be decreased by 10 % but the biomass concentration would be doubled. This is interesting for practice because a higher biomass concentration is beneficial for harvesting and the loss of biomass yield possibly is compensated by improved economics of down-stream processing of the biomass.

Specific growth rate and biomass concentration

The specific growth rate varied between replicates in the two independent PBR experiments. This variation caused opposite changes in dry weight concentration, and, as a result, volumetric productivities, and biomass yields on light energy were comparable. These data confirmed experimental conditions were close to the optimal point of biomass productivity in terms of both, specific growth rate, and resulting biomass concentration. Takache et al. (2010) showed that a 15 % variation of specific growth rate around the optimal value had a minor influence on corresponding maximal biomass yield.

Both PBR systems were equipped with a blue-red LED panel but the red/blue ratio (Table 6.2) was not the same in both systems even though the overall incident PFD was comparable but subject to changes: although the PFD was set to $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ it

varied in practice from $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 6.1). But this variation is accounted for in the calculation of the biomass yield on light energy (equations 6.4-6.6). The difference in red/blue ratio could have caused experimental variations.

A turbidostat-operated PBR will, by definition, keep the turbidity of a culture constant. The turbidity can be expressed as product of dry weight and optical cross section of algae biomass (Table 6.2). A constant turbidity also results in a comparable light gradient in a similar system. A comparable light gradient is confirmed because the absorptive area per PBR volume stayed constant between the biological duplicates for all experimental conditions (Table 6.2). Comparing these data with the measured incident PFD suggested that small variations in incident PFD did not influence the overall light gradient in the PBR. Furthermore, the volumetric productivity for each biological duplicate was constant, which indicates that experimental conditions were similar.

Table 6.2 Specific growth rate, dry weight, average dry weight specific absorption coefficient, and the product of dry weight and average dry weight specific absorption coefficient, volumetric productivity and measured red/blue ratio of the light source for reference and flashing light experiments shown for two independent PBR runs (PBR 1, PBR 2)

#		μ	c_x	\bar{a}_{dw}^*	$c_x \times \bar{a}_{dw}^*$	$\mu \times c_x$	red/blue
		h^{-1}	$g_{bm} L^{-1}$	$m^2 g_{bm}^{-1}$	$m^2 L^{-1}$	$g_{bm} L^{-1} h^{-1}$	-
<i>PFD₀ = 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$, PFD_{out} = 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, continuous light</i>							
1	PBR 1	0.054±.002	0.343±.009	0.258	0.09	0.019	0.58
2	PBR 2	0.047±.002	0.401±.013	0.238	0.10	0.019	0.65
<i>PFD₀ = 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$, PFD_{out} = 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, continuous light</i>							
3	PBR 1	0.031±.001	0.324±.012	0.227	0.07	0.010	0.55
4	PBR 2	0.039±.001	0.282±.018	0.237	0.07	0.011	0.61
<i>PFD₀ = 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$, PFD_{out} = 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $\Phi = 0.5$, $f = 100\text{Hz}$</i>							
5	PBR 1	0.034±.001	0.407±.010	0.272	0.11	0.014	0.58
6	PBR 2	0.029±.000	0.409±.023	0.256	0.10	0.012	0.65
<i>PFD₀ = 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$, PFD_{out} = 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $\Phi = 0.5$, $f = 10\text{Hz}$</i>							
7	PBR 1	0.021±.001	0.450±.009	0.232	0.10	0.009	0.58
8	PBR 2	0.022±.001	0.394±.009	0.235	0.09	0.009	0.65
<i>PFD₀ = 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$, PFD_{out} = 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$, $\Phi = 0.5$, $f = 1\text{Hz}$</i>							
9	PBR 1	0.028±.001	0.366±.004	0.261	0.10	0.010	0.58
10	PBR 2	0.019±.002	0.431±.005	0.235	0.10	0.008	0.65

The standard deviations of the specific growth rate and the dry weight were calculated from the three values measured during the last 72 h of steady state.

Absorption characteristics and acclimation state

The PFD at the surface of the PBR was sub-saturating with $220 \mu\text{mol m}^{-2} \text{s}^{-1}$. The PFD leaving the PBR was just enough to support net photosynthesis and, therefore, the average PFD along the light path was reduced to a growth limiting PFD.

The absorption coefficient (\bar{a}_{dw}^* , Table 6.2, Figure 6.2a) in reference 1 experiment was $0.248 \text{m}^2 \text{g}_{bm}^{-1}$, which is similar to reference 2 experiment ($\bar{a}_{dw}^* = 0.232 \text{m}^2 \text{g}_{bm}^{-1}$). Vejrazka et al (2011) reported an absorption coefficient of $0.233 \text{m}^2 \text{g}_{bm}^{-1}$ for low light acclimated algae and an absorption coefficient of $0.127 \text{m}^2 \text{g}_{bm}^{-1}$ for high light acclimated algae; consequently, algae in reference 1 and 2 experiment responded to light limitation and maximized their absorptive area. This is in agreement with Takache et al. (2010) who observed a progressive reduction of pigmentation and absorption coefficient with increase in PFD for the same algae.

A similar result, which is a large absorption coefficient, was obtained under flashing light. The absorption coefficient was similar for each flash frequency tested, and, therefore, algae were low light acclimated at all frequencies (Figure 6.2a).

The large absorption coefficient under flashing light showed algae were light limited. And, light limitation under flashing light is also indicated by a specific growth rate (Table 6.2) below the maximal value; the maximal specific growth rate of *C. reinhardtii* is 0.13h^{-1} (Vejrazka et al., 2011).

Light acclimation mechanisms are versatile, not only the average absorption coefficient can be altered but also carotenoid to chlorophyll ratio. For this reason we plotted the dry weight specific absorption coefficient normalized to the value measured at 680 nm in Figure 6.2b. All curves in Figure 6.2b overlap and we conclude that the carotenoid to chlorophyll ratio did not change. Takache et al. (2010) showed a similar result for a wide range of PFDs ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$); as long as full light attenuation was maintained then contribution of photoinhibition, which results in an increase of carotenoid to chlorophyll ratio, was low.

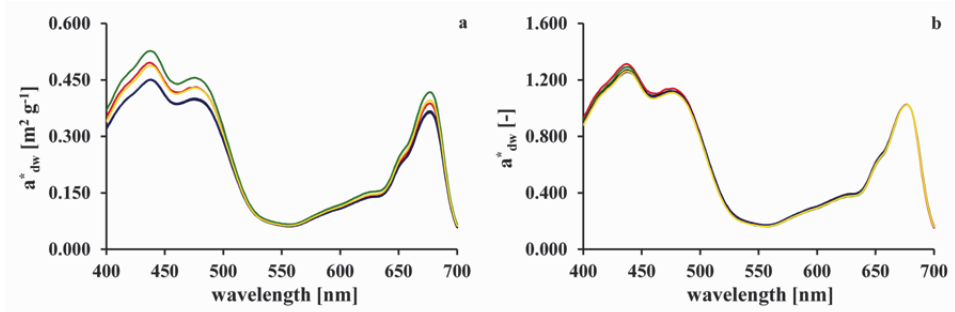


Figure 6.2: (a) wavelength-dependent dry weight specific absorption coefficient, (b) wavelength dependent dry weight specific absorption coefficient normalized to the value at 680 nm (chlorophyll absorption maximum), each line represents the average for the biological duplicate — reference 1 — reference 2 — 100 Hz — 10 Hz — 1 Hz

Conclusions

We showed that L/D cycles of 1 Hz and 10 Hz resulted in a 10 % decrease in biomass yield in comparison to no dark zone. Full light integration was reached at a flash frequency of 100 Hz.

Our data demonstrates that the light regime perceived by algae in a PBR system is an important parameter in PBR design and engineering because it can increase operation efficiency of the whole algae production process: If a well-mixed ($f \geq 1$ Hz), short light path PBR is operated under sub-saturating incident PFDs then a dark zone of maximally half the length of the light path is feasible with minor decrease in biomass yield and volumetric productivity. The benefit of the dark zone is an increase in biomass concentration, which is interesting for practice because a higher biomass concentration is beneficial for harvesting.

Appendix

Light measurement

The reference position for the spectroradiometer measurement was an arbitrarily chosen but fixed position at the rear side of the photobioreactor (PBR) cuvette. The spectral distribution was measured without the cuvette present in intervals ($\Delta\lambda$) of 1 nm as spectral photon flux density ($PFD_{ref}(\lambda)$, $\mu mol m^{-2} s^{-1} nm^{-1}$). The spectral measurement was normalized for the PAR range to obtain the normalized emission spectrum ($E_{n,PAR}(\lambda)$, nm^{-1}) using equation (6.7).

$$E_{n,PAR}(\lambda) = \frac{PFD_{ref}(\lambda)}{\sum_{\lambda=400}^{700} PFD_{ref}(\lambda) \cdot \Delta\lambda} \quad (6.7)$$

The absolute spectrum of the incident photon flux density (PFD) on the PBR surface ($PFD_0(\lambda)$) was calculated by multiplying the incident PFD (PFD_0) with the normalized emission spectrum ($E_{n,PAR}(\lambda)$).

The PAR quantum sensor measurements at the PBR surface and the Avantes spectroradiometer (SRM) measurements at the reference position determined under continuous light were correlated with each other (cf , equation (6.8)).

$$cf = \frac{\sum_{\lambda=400}^{700} PFD_{ref}(\lambda) \cdot \Delta\lambda}{PFD_0} \quad (6.8)$$

In this way, the SRM measurements could be used to determine the incident, time-averaged PFD at the PBR surface under flashing light ($PFD_{0,L/D}$, $PFD_{0,L/D}(\lambda)$) for which the LiCor sensor could not be used. The incident, time-averaged PFD ($PFD_{0,L/D}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$, equation (6.9)) at the PBR surface was calculated based on the reference spectrum for flashing light ($PFD_{ref,L/D}(\lambda)$, $\mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$).

$$PFD_{0,L/D} = \frac{\sum_{\lambda=400}^{700} PFD_{ref,L/D}(\lambda) \cdot \Delta\lambda}{cf} \quad (6.9)$$

The normalized emission spectrum for flashing light () was also calculated according to equation (6.7) but with the values for flashing light. Finally, the absolute spectrum of the incident, time-averaged PFD on the PBR surface ($PFD_{0,L/D}(\lambda)$, $\mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$) was calculated by multiplying the total incident, time-averaged PFD ($PFD_{0,L/D}$) with the normalized emission spectrum under flashing light ($E_{n,PAR,L/D}(\lambda)$). By dividing the incident, time-averaged PFD ($PFD_{0,L/D}$) with the duty cycle incident flash intensity could be calculated ($PFD_{0,L}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$, Table 6.1).

The light transmission ($T(\lambda)$, equation (6.10)) during the cultivations was verified with the SRM measurements: prior to inoculation a blank spectral PFD ($PFD_{ref,PBR}^0(\lambda)$) was measured (with PBR cuvette, growth medium, and mixed by aeration). The blank measurement and the measurement during the cultivation ($PFD_{ref,PBR}^1(\lambda)$) was used to calculate light transmission ($T(\lambda)$, equation (6.10)) at each wavelength interval.

$$T(\lambda) = \frac{PFD_{ref,PBR}^1(\lambda)}{PFD_{ref,PBR}^0(\lambda)} \quad (6.10)$$

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Chapter 7

General discussion

Introduction

Microalgae have a potential to be a feedstock for a biobased economy because of their ability to use sunlight as an energy source and carbon dioxide as a carbon source. The production of low value compounds (e.g. bulk chemicals, biofuels) depends on very efficient large-scale cultivation systems such as open ponds or photobioreactors. The first step of any algae cultivation process, independent from the product, is an efficient accumulation or production of the biomass itself, whereas it is desired to reach a biomass concentration as high as possible. A high biomass concentration ($> 1 \text{ g L}^{-1}$) in a photobioreactor (PBR) results in a light gradient because of light attenuation by the algae and possibly a dark zone. Due to mixing algae will move through the light gradient and experience fluctuating light. In the presence of a dark zone algae will experience light/dark (L/D) cycles.

The significance of fluctuating light and L/D cycles in PBRs

In chapter 2 the impact of mixing (L/D cycles) on photobioreactor (PBR) productivity was discussed. One result of an increased mixing is a better mass transfer of oxygen into the gas phase and carbon dioxide into the liquid phase. In other words photosynthetically produced oxygen that can inhibit algae growth is removed more rapidly. Therefore, an increased mixing rate (L/D cycling) can increase PBR productivity by reducing dissolved oxygen concentration.

Photobioreactor studies do not always report information about the size of the dark zone or if there was a dark zone at all, which would be important for evaluating the influence of flashing light on productivity. In this context it is important to design suitable reference experiments: no dark zone versus dark zone to truly evaluate the impact of flashing light on productivity or yield.

Photobioreactor experiments that reported about dark zones showed that under over-saturating incident PFDs the highest efficiency was reached if algae experienced L/D cycles (Hu and Richmond, 1996; Degen et al., 2001; Zijffers et al., 2010). In contrast to these experiments, Takache et al. (2010) showed for different incident photon flux densities (growth limiting to over-saturating) in a torus PBR and a cylindrical PBR (with a fixed mixing rate) that the maximal productivity and yield were achieved if the photon

flux density (PFD) leaving the PBR was at the compensation point of photosynthesis. In other words, light was fully attenuated but no dark zone was present and algae were experiencing fluctuating light but no L/D cycles.

In conclusion L/D cycles are not always necessary to achieve the optimal productivity of a given PBR system. The influence of full light attenuation will be discussed in the next paragraph to set the results from the PBR experiments under L/D cycles with minimized light attenuation (chapter 3) into perspective.

In this thesis one study was done under full light attenuation (chapter 6). To get further inside into the influence of full light attenuation on biomass yield we performed another PBR experiment at a low incident PFD ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$). The PFD leaving the PBR was $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ (minimized light attenuation) and $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ (full light attenuation). The light source was a red LED light (peak at 630 nm) and the PBR was operated as a turbidostat (more details about the PBR operation procedure can be found in chapter 4).

The cultivation with a minimized light attenuation resulted in a biomass yield of $0.99 \pm 0.08 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$, which was higher than for the cultivation with full light attenuation: $0.80 \pm 0.04 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$. The biomass yield for the minimized light attenuation was similar to the maximal net oxygen yield on light energy measured in the BOM (P-I curve, chapter 4), thus optimal. The difference between the two experiments can be explained by the influence of maintenance respiration (chapter 3): for PFDs less than $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ the relative fraction of absorbed light needed for maintenance increases. And, during full light attenuation algae were exposed to the optimal PFD $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ only if they reached the light exposed PBR surface. Contrary to the decrease in biomass yield is the increase in volumetric productivity from $0.0056 \pm 0.0003 \text{ g}_{\text{bm}} \text{ L}^{-1} \text{ h}^{-1}$ (minimized light attenuation) to $0.0103 \pm 0.0008 \text{ g}_{\text{bm}} \text{ L}^{-1} \text{ h}^{-1}$ (full light attenuation) due to a much higher biomass concentration in case of full light attenuation, which was needed to intercept 90 % of the incoming PFD.

If the incident PFD is increased to $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ (chapter 6) then the biomass yield under full light attenuation decreased to $0.60 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$. For a further increase in incident PFD to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (chapter 3) we have only information about the biomass yield for a situation with minimal light attenuation: $0.25 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$. In order to get information about the influence of full light attenuation on biomass yield we can look at Janssen et al. (2000): they showed in a similar study with the alga *Dunaliella*

tertiolecta growing at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ that under full light attenuation the biomass yield doubled in comparison to minimal light attenuation. Assuming we can extrapolate these results to our study (chapter 3) we would have reached a biomass yield of $0.50 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$. In this way, the reference situation for an over-saturating incident PFD is adapted to a more realistic value because in a large-scale PBR full light attenuation will be present. Additionally, with increase in incident PFD the volumetric productivity will increase as well. At this point we can conclude that full light attenuation is important for the biomass yield.

If, additional to full light attenuation L/D cycles are applied then we showed in chapter 6 (full light attenuation) that for low frequencies (1 Hz to 10 Hz) the effect on the biomass yield was minor (10 % decrease on average) and only at 100 Hz full light integration was reached. A similar result was achieved for an over-saturating, incident PFD (chapter 3): full light integration was only observed at 100 Hz. At 5 Hz and 10 Hz partial integration was observed if compared to a reference situation with minimal light attenuation during the flash. But, the situation changes if the results are compared to the hypothetical reference situation with full light attenuation during the flash ($0.50 \text{ g}_{\text{bm}} \text{ mol}_{\text{ph}}^{-1}$). In this case the biomass yield under 5 Hz and 10 Hz L/D cycles will be in the same range as for the situation without L/D cycles. Therefore, L/D cycles in the range of 1 Hz to 10 Hz do not have a major impact on biomass yield and volumetric productivity in comparison to a situation without L/D cycles. On the other hand, a clear advantage of L/D cycling is a higher allowable biomass concentration, which will improve the efficiency of downstream processing of the microalgae cultures.

The following section will put these conclusions in relation to frequencies that can be found in large-scale PBRs and discuss its implications on PBR operation.

Implications of L/D cycles on photobioreactor design and operation

In general L/D cycles have a positive impact on photosynthetic rate and biomass yield if the flash frequency is at least 1 Hz as shown in this thesis. Slower L/D cycles will result in a decrease in biomass yield (Janssen, 2002). Therefore, it is practical to have knowledge about the hydrodynamics of the PBR system that will be used for the cultivation. This knowledge can be obtained by CFD modeling (Moberg et al., 2011; Perner-Nochta and Posten, 2007; Pruvost et al., 2006, 2008; Wu et al., 2009) or by experimental analysis with e.g. the CARPT method (Luo et al., 2003) or PIV method (Pruvost et al. 2002a).

So far, a limited number of studies on the order of magnitude of L/D cycles in common PBR systems are available. For a tubular PBR ($d = 40$ mm) L/D cycles with frequencies of 0.2 - 3.1 Hz were reported by Perner-Nochta and Posten (2007). Furthermore, Moberg et al. (2011) showed maximal L/D cycle frequencies in a half-filled, tubular PBR ($d = 24$ mm) of 0.5 - 2.7 Hz, whereas the frequency decreased with increase in tube diameter. And, it was also shown that a 10 fold increase in liquid flow (0.1 m s^{-1} to 1 m s^{-1}) resulted in a 6 fold increase in L/D cycle frequency. Higher frequencies could be achieved by the introduction of static mixers: 3 - 25 Hz, but behind the mixing element the flow quickly lost these L/D cycle frequencies (Perner-Nochta and Posten, 2007). Additionally, Wu et al. (2009) showed for a spiral tubular PBR a 10 times higher pressure drop per meter of PBR in comparison to a conventional tubular PBR. Therefore, the gain in mixing is paid back by a higher energy input for pumping. Another option to induce a more directed flow in a tubular PBR is to create a swirling decaying flow by a tangential inlet (Pruvost et al., 2002b). However, for this system no information about actual L/D cycle frequencies is available. But, light absorption in the swirling flow was improved due to a more uniform light exposure.

No L/D cycle frequencies are reported for flat panel PBRs. One study (Luo et al., 2003) investigated the mixing and L/D cycling in a draft tube column and split tube column. Even though these tubes are not rectangular as flat panel PBRs and have a much longer light path, they might give an indication of the L/D cycle frequencies to be expected in a flat panel PBR because both work on the airlift principle. Luo et al. (2003) found frequencies on three different scales: 0.1, 1 and 10 Hz, whereas the lowest was due to the circulation between riser and downcomer, which might not be relevant for flat panel PBRs. The two lower frequencies (0.1 Hz and 1 Hz) were due to spiral movement and turbulence. The turbulence induced L/D cycle frequency (10 Hz) is very interesting and might be even higher in flat panel PBRs due to a much shorter light path.

To summarize, the studies on tubular PBRs and column PBRs showed maximal L/D cycle frequencies in the range of 1 - 10 Hz. As discussed in the previous sections, at these L/D cycles the influence on biomass yield and volumetric productivity is minor.

This has two implications: first, CFD modeling could be used to determine the lowest energy input in terms of mixing in order to establish a flashing light regime with frequencies of 1 Hz because lower frequencies would lead to a decrease in biomass yield (Janssen, 2002); second the duty cycle specific, incident PFD, i.e. duty cycle times incident PFD, is of importance because this value (based on a given incident PFD) will

define the minimal duty cycle (or maximal dark zone). The information about the dark zone can be translated with the help of a light attenuation model into an optimal biomass concentration for PBR operation. The criteria to set the optimal duty cycle specific, incident PFD will be discussed in the next paragraph.

In chapter 3 the P-I curve under continuous light for *Chlamydomonas reinhardtii* was discussed. The main result was that the highest net oxygen yields on light energy (comparable to biomass yield on light energy) were reached at PFDs from 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Below this range the yield was reduced due to maintenance requirements and above this range the yield was reduced due to heat dissipation of excess absorbed light energy. The same trend was observed under flashing light with an over-saturating PFD while applying different duty cycles: the optimum duty cycle was 0.1, which equaled an incident, time-averaged PFD of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Therefore, it can be argued that for PBR operation the duty cycle specific, incident PFD should be in the same range where the optimum of the P-I curve is located in order to achieve the highest yield. With this knowledge and data from the PBR experiments (see section: “The significance of fluctuating light and L/D cycles in PBRs”) the following rule of thumb can be formulated: If the duty cycle specific, incident PFD is in the optimal range (based on P-I curve) then the biomass yield under flashing light (1 - 10 Hz) will be similar to the yield under continuous light with the flash PFD and full light attenuation (i.e. a PFD leaving the PBR at the level of compensation point for photosynthesis). This rule of thumb implies that there are limits for the size of the dark zone as seen e.g. in chapter 6: the biomass yield for 1 Hz and 10 Hz was on average 10 % lower than for continuous illumination with the flash PFD. A further increase in dark zone (decrease in duty cycle specific, incident PFD) would probably have resulted in a further decrease in yield due to maintenance respiration losses.

Based on these results an operation window of duty cycles can be defined, which could be a duty cycle range of e.g. $\pm 20\%$ of the optimum. Based on this window and growth characteristics of the algae the PBR can be diluted once or twice a day depending on the following downstream process. A more frequent dilution is probably not technological reasonable because the biomass suspension has to be stored and depending on the climate conditions also cooled, which would require additional energy input.

The relation between the results under L/D cycles and continuous light found in chapter 4 can give a guideline which duty cycles are suitable for maximal output. Therefore, it is of importance to know the P-I curve characteristics of both, oxygen production as a

measure of growth and oxygen yield on light energy as a measure of efficiency. Based on the yield optimum in the P-I curve the duty cycle range for PBR operation can be determined. Consequently, to apply this optimal range of duty cycles the (future) operation mode of PBRs should be done as turbidostat because the light regime inside the PBR can be controlled in turbidostat mode only. In this way it is possible to adjust the light zone and dark zone to the right size.

Finally, the question remains which incident PFD should be applied. An over-saturating, incident PFD (full sun light exposure, chapter 3) will lead to lower yields but much higher biomass concentrations because a greater dark zone can be allowed and a higher volumetric productivity can be gained. A saturating, incident PFD (light dilution by placing PBR vertically in each other's shade, chapter 6) will result in higher yields but lower biomass concentrations because of a smaller dark zone. Practically, the scenario with the higher biomass concentration might be chosen because further processing steps will become more efficient.

Potential of the dynamic model

The presented model (chapter 5) describes electron production due to light absorption in flashing light. Electrons accumulate during the light phase and aid growth during the dark part of the L/D cycle. Photosynthetic oxygen production was coupled to electron consumption to describe photosynthesis. The model and the underlying mechanism of electron accumulation could describe the measured oxygen production rates well.

However, data was measured within a narrow range of flash PFDs (1090-1350 $\mu\text{mol m}^{-2} \text{s}^{-1}$, see chapter 3 for explanation), therefore, it is not clear how well it will describe oxygen production for different flash intensities. In addition, the model was developed and validated for flash frequencies above 1 Hz. It might not be able to describe algae growth and oxygen production below this frequency. A recently proposed model from García-Camacho et al. (2012) might be suitable to describe oxygen production below 1 Hz. Furthermore, the energy dissipation rate was based on a Monod-type kinetic with a variable maximal rate. This relation should be further investigated because energy dissipation rates were not equally well reproduced for each set of measurements.

In general, this model could be the basis of an overall growth model. For this purpose it should be possible to determine the necessary model parameters in simple measurements. One way to do this could be a P-I curve measurement (chapter 4). P-I curves are measured relatively fast in comparison to the specific growth rate. The P-I

curve measurement must include light respiration (see chapter 4), which is necessary to determine gross oxygen yield and energy dissipation rate. From these data the maximal electron consumption rate, which is based on maximal gross oxygen production rate and the maximal gross yield of electrons on photons can be estimated. Based on respiration measurements the correlation between net oxygen production and respiration can be established and maintenance respiration can be determined as well. The only two fitting parameters that remain unknown are the half-saturation constants for electron consumption and light energy dissipation. With an adequate amount of P-I curve data points (> 10) these two parameters could be fitted to the measured data.

Furthermore, it might be sufficient to determine the P-I curve for low light acclimated algae because PBR experiments in this thesis showed that algae grown under flashing light and fluctuating light were low light acclimated (maximized absorption cross section).

Figure 7.1 shows to P-I curve and the simulation based on the hyperbolic tangent model as well as the simulation based on the dynamic model developed in Chapter 4. The dynamic model can predict the general trend but the hyperbolic tangent model predicts the saturation behavior between $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ better. This discrepancy is probably due to the prediction of the energy dissipation rate (Figure 7.2), which is underestimated if photosynthesis changes from being light-limited to light saturated. Therefore, other possibilities that connect the energy dissipation rate to e.g. internal metabolites or “true” reduction state of the photosynthetic electron transport chain might be investigated. If the prediction of the dissipation rate can be fine-tuned then the P-I curve measurement will be a valuable tool to determine the model parameters because the model can describe both, growth under continuous light and growth under flashing light.

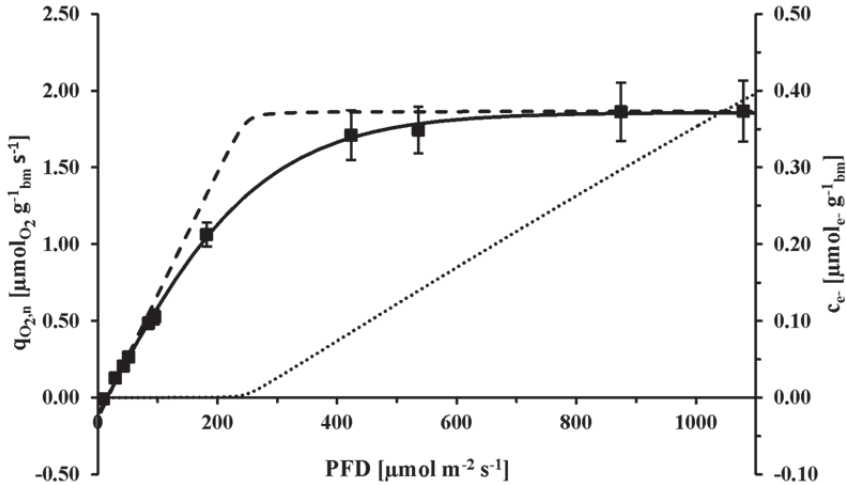


Figure 7.1: Measured net oxygen production rate (■) and simulated steady state electron concentration (···) versus photon flux density (continuous light), — simulated net oxygen production rate based on hyperbolic tangent model (chapter 3) --- simulated net oxygen production rate based on dynamic model (chapter 4).

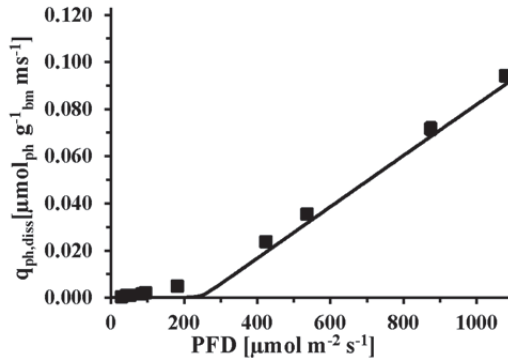


Figure 7.2 Photon dissipation rate versus photon flux density for continuous light, ■ calculation based on measured values, — simulation based on dynamic model (chapter 4)

The model was developed based on experimental results obtained under simulated L/D cycles with minimal light attenuation and it is a good basis to predict the dynamic response of algae in a PBR. Specifically, it can be used to simulate algal growth under full light attenuation with different scenarios of L/D cycles and to compare these results to measured data.

In the section “Implications of L/D cycles on photobioreactor design and operation” it was discussed that no information is available about L/D cycles in flat panel PBRs but there is potential that these L/D cycles might be in the range of 10 Hz or even higher. The biologic model could be used to determine the order of magnitude of L/D cycles that can be found in a flat panel PBR. For this purpose growth experiments in a turbidostat

operated PBR can be used, whereas a reference experiment without a dark zone and additional experiments with well-defined dark zones need to be done. Additionally, the influence of different mixing rates (i.e. gas supply rates) can be studied as well. The measured biomass yield can be fit to the dynamic model with the L/D cycle settings as the fitting parameters. The model output is the net oxygen production rate that can be translated into a growth rate with knowledge about growth stoichiometry and elemental composition. And, the growth rate is the basis for the biomass yield calculation. If the estimated frequencies exceed 10 Hz then CFD modeling can be used as an additional tool to further investigate these L/D cycles and their dependency on the gassing rate.

Conclusion

The following overall conclusions can be drawn from this thesis:

- Algae growing under limiting, duty cycle specific, incident PFD (i.e. duty cycles times incident PFD) have a maximized optical cross section
- Optimal duty cycle specific, incident PFD for PBR operation can be deduced from P-I curve measurements, which include light respiration (optimum *Chlamydomonas reinhardtii*: 100 - 200 $\mu\text{mol s}^{-1} \text{m}^{-2}$)
- Light/dark cycle frequencies of 1-10 Hz, which can be achieved in practice, have a minor impact on biomass yield and volumetric productivity
- PBR can be operated with a dark zone with minor influence on biomass yield but a major gain in biomass concentration
- Light integration can be explained by an electron storage pool that fills during the flash and is used during the dark

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Summary

Microalgae are promising organisms for a biobased economy as a sustainable source of food, feed and fuel. High-density microalgae production could become cost effective in closed photobioreactors (PBR). Therefore, design and optimization of closed PBRs is a topic of ongoing research in both academic and industrial environment.

Mixing in dense algae cultures causes light/dark (L/D) cycles of different magnitudes exposing algae to flashing light. It is often said that due to a flashing light effect, productivity of a PBR can be increased. In this thesis the flashing light effect is systematically investigated and the result is a mechanistic model that can predict microalgae growth under different flashing light regimes.

The review of existing literature about L/D cycle experiments in **Chapter 2** provides the theoretical background of the flashing light effect (L/D cycles) and discusses possibilities to improve PBR productivity by its application. It is concluded that PBR performance can be optimized by maximizing photosynthetic rate and biomass yield on light energy based on increased or controlled mixing and, thus, L/D cycling. It is unlikely to achieve maximal enhancement based on L/D cycles because of the fast mixing required: specific growth rate measurements in well-controlled, lab-scale PBRs suggest a minimal flash frequency of 14 Hz - 24 Hz combined with short flash times (< 20 ms) to achieve a maximal enhancement.

The application of flashing light alone in an artificially illuminated PBR has a limited effect on PBR performance, consequently, continuous (sun) light should be preferred. Further optimization strategies can be developed based on mechanistic models that describe the influence of L/D cycles on algae productivity as will be shown later in this thesis (Chapter 5).

In **Chapter 3**, photosynthetic efficiency and growth of the green microalga *Chlamydomonas reinhardtii* were measured using LED light to simulate light/dark cycles ranging from 5 to 100 Hz at a light/dark ratio of 0.1 and a flash photon flux density (PFD) of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Light flashing at 100 Hz yielded the same photosynthetic efficiency and specific growth rate as cultivation under continuous illumination with the same time-averaged PFD, which is called full light integration. The efficiency and growth rate decreased with decreasing flash frequency. At all frequencies, the rate of linear electron transport during the flash was higher than during maximal growth under continuous light, suggesting

storage of reducing equivalents during the flash, which are available during the dark period. In this way the dark reaction of photosynthesis can continue during the dark time of an L/D cycle. This is a possible explanation for the mechanism behind the flashing light effect.

Another parameter that describes an L/D cycle besides frequency, is the duty cycle, it determines the time fraction algae spend in the light. In **Chapter 4** the influence of different duty cycles on oxygen yield on absorbed light energy and photosynthetic oxygen evolution was investigated. Net oxygen evolution of *Chlamydomonas reinhardtii* was measured for four duty cycles (0.05, 0.1, 0.2 and 0.5) in a biological oxygen monitor. Over-saturating light flashes were applied in a square-wave fashion with four flash frequencies (5, 10, 50, 100 Hz). Algae were pre-cultivated in a turbidostat and acclimated to a low photon flux density (PFD).

A photosynthesis-irradiance curve was measured under continuous illumination and used to calculate the net oxygen yield, which was maximal between a PFD of 100 and 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Net oxygen yield under flashing light was proven to be duty cycle dependent: the highest yield was observed at a duty cycle of 0.1 (i.e. a time-averaged PFD of 115 $\mu\text{mol m}^{-2} \text{s}^{-1}$). At lower duty cycles maintenance respiration reduced net oxygen yield. At higher duty cycles photon absorption rate exceeded the maximal photon utilization rate and, as a result, surplus light energy was dissipated as heat, which lead to a reduction in net oxygen yield. This behavior was identical with the observation under continuous light.

Understanding photosynthetic growth in dynamic light regimes is crucial to develop models that can predict PBR productivities under continuous and flashing light. Therefore, the objective of **Chapter 5** was to develop and validate a mechanistic model that describes photosynthetic net oxygen evolution under flashing light based on biomass specific light absorption rate and light dissipation rate of excess absorbed light.

The model describes photosynthetic oxygen evolution based on the availability of reducing equivalents (electrons), which result from the light reactions. Electrons are accumulated during the flash and serve as a pool for carbon dioxide fixation during the dark, which leads to partial or full light integration. Both, electron consumption rate and energy dissipation rate are based on a Monod-type kinetic.

The underlying assumption of an electron pool seems correct and its filling and emptying is depending on the flash time. In general, with increase in flash time the energy dissipation rate increased as well. And, simulations showed that if the dark time

between flashes is not sufficiently long then the pool will not be completely empty and is responsible for a high energy dissipation rate. The measured oxygen production rates were described well, but the description of the energy dissipation rate will need further investigation.

Not only L/D cycles but also fluctuating light that algae experience while moving through the light gradient will influence PBR productivity. In **Chapter 6** the combined effect of L/D cycles and fluctuating light on biomass yield on light energy was studied.

For this purpose we used controlled, short light path, laboratory, turbidostat-operated PBRs equipped with a LED light source for square-wave L/D cycles with frequencies from 1 Hz to 100 Hz. Biomass density was adjusted that the PFD leaving the PBR was equal to the compensation point of photosynthesis ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Algae were acclimated to a sub-saturating incident PFD of $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ for continuous light. Using a duty cycle of 0.5, we observed that L/D cycles of 1 Hz and 10 Hz resulted on average in a 10 % lower biomass yield, but L/D cycles of 100 Hz resulted on average in a 35 % higher biomass yield than the yield obtained in continuous light.

The results show that the interaction of L/D cycle frequency, culture density and incident PFD lead to certain PBR productivity. Hence, appropriate L/D cycle frequency setting by mixing and dark zone setting by biomass concentration can optimize PBR productivity. And, reduce the effect that a dark zone exposure impinges on biomass yield in microalgae cultivation.

The last chapter is a general discussion (**Chapter 7**) that places the results of the thesis into context for PBR operation. It is discussed that L/D cycle frequencies of 1-10 Hz, which can be achieved in practice, have a minor impact on biomass yield and volumetric productivity. But, the PBR can be operated with a dark zone and a major gain in biomass concentration can be achieved. However, the size of the dark zone is limited by the incident PFD. The incident PFD times the relative size of the photic zone should be in the same range where the optimal yield under continuous light can be found based on a P-I curve measurement. The photic zone is then defined as the volume with a PFD above the compensation point divided by the total volume.

With simulations based on the dynamic model we could show that light integration can be explained by an electron storage pool that fills during the flash and is used during the

dark. Furthermore, the dynamic model could be used to predict PBR productivities based on real fluctuating light regimes observed in photobioreactors.

Samenvatting

Microalgen zijn veelbelovende organismen voor een 'biobased' economie. Ze kunnen gebruikt worden als een duurzame bron voor voedsel, vee- en visvoerders en biobrandstoffen. Microalgen kunnen in de toekomst mogelijk kosteneffectief geproduceerd worden in fotobioreactoren bij een hoge celdichtheid. Om deze reden is het ontwerp en de optimalisatie van gesloten fotobioreactoren onderwerp van lopend onderzoek in zowel de academische als industriële omgeving.

Mengen van dichte algen culturen resulteert in licht/donker (L/D) cycli van verschillende lengte en de algen worden aan dit knipperende (intermitterende) licht blootgesteld. Vaak wordt gesteld dat de productiviteit van fotobioreactoren kan worden verhoogd door dit zogenaamde knipperlicht effect. In dit proefschrift wordt het knipperlicht effect systematisch onderzocht en het resultaat is een mechanistisch model dat de groei van microalgen kan voorspellen onder verschillende L/D cycli.

Een literatuurstudie over L/D cycli in **hoofdstuk 2** geeft de theoretische achtergrond van het knipperlicht effect en de mogelijkheden worden besproken om de productiviteit van fotobioreactoren te verbeteren door de toepassing van dit effect. Inderdaad zou de productiviteit kunnen worden geoptimaliseerd door versneld en/of gecontroleerd mengen van fotobioreactoren die met zonlicht worden belicht. Dit zou kunnen resulteren in snelle en gecontroleerde L/D cycli en het maximaliseren van de fotosynthese snelheid en biomassa opbrengst op licht-energie. Het is echter onwaarschijnlijk dat de maximale verhoging op basis van het knipperlicht effect in de praktijk te bereiken is vanwege de wel zeer snelle menging die hiervoor nodig is. Metingen van de specifieke groeisnelheid onder flitslicht in goed gecontroleerde lab-schaal fotobioreactoren duiden op een minimale flits frequentie van 14 Hz tot 24 Hz in combinatie met een korte flits tijd (< 20 ms) die nodig is voor een maximale verbetering.

De toepassing van intermitterende belichting in een fotobioreactor die met kunstlicht wordt belicht heeft slechts een beperkt effect op de fotobioreactor productiviteit. Daarom heeft het gebruik van continu zonlicht de voorkeur. Verdere optimalisatie strategieën kunnen worden ontwikkeld op basis van mechanistische modellen die de invloed van L/D cycli op algengroei beschrijven. Dit is het onderwerp van **hoofdstuk 5** van dit proefschrift dat later zal worden besproken.

In **hoofdstuk 3** wordt eerst de fotosynthetische efficiëntie en de groei van de groene microalg *Chlamydomonas reinhardtii* beschreven zoals deze gemeten is onder

verschillende L/D cycli met behulp van flitsend LED licht (LED = light emitting diode). De L/D cycli varieerden van 5 tot 100 Hz bij een licht/donker verhouding van 0.1 en een fotonen fluxdichtheid (FFD) van $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Intermitterend licht van 100 Hz leverde dezelfde fotosynthetische efficiëntie en specifieke groeisnelheid op als continue belichting van dezelfde tijdsgemiddelde FFD. Dit wordt ook wel volledige lichtintegratie genoemd. De efficiëntie en groei daalden met afnemende frequentie. Bij alle frequenties was de snelheid van het lineaire elektron transport tijdens de lichtflits hoger dan tijdens maximale groei onder continu licht. Dit wijst op de opslag van reducerende equivalenten tijdens de flits die weer beschikbaar zijn tijdens de daaropvolgende donkere periode. Op deze wijze kunnen de 'donker reacties' van de fotosynthese gewoon doorgaan. Dit is een mogelijke verklaring voor het mechanisme achter het knipperlicht effect.

Een andere parameter die de L/D cycli karakteriseert is de relatieve 'inschakeltijd'. Dit is de fractie van de tijd die de algen door brengen in het licht. In **hoofdstuk 4** wordt de invloed beschreven van verschillende inschakeltijden op de zuurstof opbrengst op geabsorbeerde lichtenergie. Ook werd de absolute snelheid van de fotosynthetische zuurstof productie onderzocht. De netto zuurstof productie van *Chlamydomonas* werd bepaald in een biologische zuurstofmonitor onder vier relatieve inschakeltijden: 0.05, 0.1, 0.2 en 0.5. Over-verzadigende lichtflitsen werden toegepast volgens blokvormige L/D cycli met vier flitsfrequenties: 5, 10, 50 en 100 Hz. De algen werden vooraf gekweekt in een turbidostaat en geacclimatiseerd aan een lage fotonen fluxdichtheid.

In een referentie experiment werd een fotosynthese-instraling curve gemeten onder continue belichting en deze werd gebruikt om de netto zuurstof opbrengst op geabsorbeerde lichtenergie te berekenen. Deze was het grootst tussen een fotonen fluxdichtheid (FFD) van 100 en $200 \mu\text{mol m}^{-2} \text{s}^{-1}$. De netto zuurstofopbrengst onder intermitterend licht was afhankelijk van de relatieve inschakeltijd. De hoogste opbrengst werd waargenomen bij een relatieve inschakeltijd van 0.1 overeenkomend met een tijdsgemiddelde FFD van $115 \mu\text{mol m}^{-2} \text{s}^{-1}$. Bij lagere inschakeltijden was de netto zuurstof opbrengst lager ten gevolge van de ademhaling die nodig is voor cellulaire onderhoudsprocessen. Bij hogere relatieve inschakeltijden was de fotonen absorptie hoger dan de maximale verwerkingssnelheid van fotonen en als gevolg daarvan werd overtollige licht energie afgevoerd als warmte (dissipatie) wat leidde tot een verlaging van de netto zuurstof opbrengst. Deze observaties waren aldus vergelijkbaar met de observatie onder continu licht dat er een optimum FFD is tussen 100 en $200 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Een beter begrip van fotosynthese, en dus algengroei, onder intermitterend licht is cruciaal om modellen te kunnen ontwikkelen waarmee de productiviteit van fotobioreactoren voorspeld kan worden. De doelstelling van **hoofdstuk 5** was daarom het ontwikkelen en valideren van een mechanistisch model dat de netto fotosynthetische zuurstof productie beschrijft onder intermitterend licht. Dit model is ontworpen op basis van de biomassa specifieke absorptie snelheid van lichtenergie en de biomassa specifieke snelheid waarmee overtollig licht wordt afgevoerd (gedissipeerd).

Het model beschrijft de zuurstof productie op basis van de beschikbaarheid van reducerende equivalenten (elektronen), die uit water worden vrijgemaakt tijdens de 'licht reacties' van de fotosynthese. Elektronen hopen op tijdens de flits en deze dienen als een reservoir voor de omzetting van kooldioxide in suikers in het donker, de 'donker reacties' van de fotosynthese. Dit opslageffect leidt dan tot een gedeeltelijke, of volledige, licht integratie. Zowel het verbruik van elektronen in de donker reacties als wel de dissipatie van licht energie zijn beschreven met Monod kinetiek.

De onderliggende aanname van een elektron reservoir lijkt correct op basis van de validatie experimenten die uitgevoerd zijn en het vullen en legen van het reservoir is afhankelijk van de lengte van de licht flits. De dissipatie van licht energie steeg met een toename van de flits lengte. Verder is uit de simulaties gebleken dat het reservoir niet helemaal geleegd wordt als de donkere periode tussen opeenvolgende licht flitsen niet lang genoeg is en dat dit leidt tot de geobserveerde hoge dissipatie van lichtenergie. In het algemeen beschreef het model de gemeten zuurstofproductie snelheden goed, De dissipatie van lichtenergie werd echter minder goed beschreven en dit zal verder onderzocht moeten worden.

Naast licht/donker cycli (intermitterend licht, aan/uit), zal ook het fluctuerend en gradueel veranderd licht dat algen ervaring tijdens hun beweging door een fotobioreactor de productiviteit beïnvloeden. In **hoofdstuk 6** wordt het gecombineerde effect van L/D cycli en fluctuerend licht bestudeerd op basis van de gemeten biomassa opbrengst op lichtenergie. Hiervoor hebben we een laboratorium fotobioreactor gebruikt met een korte lichtweg welke bedreven werd als een turbidostaat. Deze bioreactor was voorzien van een LED lichtbron waarmee blokvormige L/D cycli konden worden gesimuleerd met frequenties van 1 Hz tot 100 Hz. De biomassa dichtheid werd continu aangepast zodanig dat de fotonen fluxdichtheid (FFD) aan de achterzijde van de fotobioreactor gelijk was aan het compensatiepunt van de fotosynthese ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$) om op deze manier een licht gradiënt te creëren.

Voor het referentie experiment onder continue belichting (geen L/d cycli maar wel licht gradiënt) werd de FFD aan het reactoroppervlak op $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ ingesteld. Dit is een FFD waarbij de fotosynthese van *Chlamydomonas* bijna verzadigd is. Een relatieve inschakeltijd van 0.5 onder L/D cycli van 1 Hz en 10 Hz resulteerde gemiddeld in een 10 % lager biomassaopbrengst in vergelijking met continu licht. L/D cycli van 100 Hz daarentegen resulteerden in een gemiddeld 35 % hogere biomassaopbrengst. De resultaten tonen aan dat de interactie van de frequentie van L/D cycli, de biomassadichtheid en de FFD aan het reactoroppervlak samen leiden tot een bepaalde productiviteit. De productiviteit van fotobioreactoren kan dus gemaximaliseerd worden door optimalisatie van L/D cycli door middel van de menging, en optimalisatie van de grootte van de donkere zone door middel van een juiste instelling van de biomassaconcentratie.

In de algemene discussie (**hoofdstuk 7**) worden de resultaten van dit proefschrift verder uitgewerkt met betrekking tot het optimaal bedrijven van fotobioreactoren. Er wordt besproken dat L/D cycli van 1 - 10 Hz, die in de praktijk worden bereikt, weinig effect op de biomassaopbrengst en op de volumetrische productiviteit hebben. De fotobioreactor kan daarentegen bedreven worden met een aanzienlijke donkere zone waardoor de biomassa concentratie verhoogd kan worden zonder veel verlies in opbrengst. De relatieve grootte van deze donkere ('niet-fotische') zone wordt begrensd door de grootte van de FFD aan het reactoroppervlak. De invallende FFD vermenigvuldigd met de relatieve omvang van de 'fotische' zone moet in hetzelfde bereik liggen als waar het optimale rendement op lichtenergie wordt gemeten in een fotosynthese-instraling curve onder continu licht. De fotische zone wordt hierbij gedefinieerd als het relatieve reactor volume met een FFD boven het compensatiepunt van de fotosynthese.

Met behulp van simulaties met het dynamische model konden we laten zien dat lichtintegratie kan worden verklaard door een elektronen reservoir dat wordt opgevuld tijdens een lichtflits en weer wordt gebruikt in het donker. Dit dynamische model kan verder worden gebruikt om de productiviteit van fotobioreactoren te voorspellen op basis van de reële licht regimes die microalgen ervaren in deze bioreactoren.

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I hope you enjoyed reading it.

Best Regards,

Carsten

Curriculum vitae



Carsten Vejrazka was born on the 8th of March, 1982 in Dresden, Germany. After finishing his highschool education at the “Bertolt Brecht Gymnasium” in Dresden, he did one year of community service taking care of elderly people. In 2001 he started to study Bioprocess Engineering at the University of Technology in Dresden. In 2007 he received the degree “Diplom-Ingenieur” (equivalent to M.Sc.).

During his studies he worked on a research project at the University of Technology in Dresden. This project was about the isolation of peptides that bind specifically to *Salmonella typhimurium*. Another research project was done in 2006 at the Max-Planck Institute for dynamics of complex technical systems. Within this project Carsten investigated the influence of cultivation strategies during influenza virus replication on virus yield. Carsten did his final thesis (Diploma thesis) at the Bioprocess Engineering Group at Wageningen University. During his Diploma thesis he investigated the growth of green microalgae in a newly developed spinning tube-in-tube photobioreactor.

In 2008 he started his Ph.D. research at the Bioprocess Engineering group at Wageningen University. The results from this research are presented in this thesis.

Training activities

Courses

Discipline specific

Bioreactor Design and Operation	(2008)
Summer School Solar H2	(2008)
Thermodynamics in Biochemical Engineering	(2008)
Advanced Course on Microbial Physiology and Fermentation Technology	(2009)

General courses

VLAG PhD week	(2008)
Teaching and supervising thesis students	(2009)
gPROMs programming	(2010)
Career Orientation	(2011)
PhD scientific writing	(2011)

Conferences

Solar H2 workshop (Uppsala, Sweden; Berlin, Germany; Tarragona, Spain)	(2011, 2010, 2009)
Sunbiopath meeting, Verona, Italy	(2011)
8th European workshop of biotechnology of microalgae, Nuthetal, Germany	(2010)
Minisymposium Algae, Wageningen, The Netherlands	(2010)
BSDL Symposium, Delft, The Netherlands	(2009)
12th Netherlands Biotechnology Congress, Ede, The Netherlands	(2008)

Optional

Preparation project proposal	(2008)
PhD Trip Japan	(2008)
PhD Trip USA	(2010)
PhD excursion to Intervet	(2008)
PhD excursion to IGV GmbH, Salata GmbH, Germany	(2009)
Brain and Game day	(2008)
Brainstorm day	(2009, 2010)

Teaching

Development of a case study for the photobioreactor design module in the course “Advanced marine biotechnology”

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