

Maximum Photosynthetic Yield of Green Microalgae in Photobioreactors

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Abstract The biomass yield on light energy of *Dunaliella tertiolecta* and *Chlorella sorokiniana* was investigated in a 1.25- and 2.15-cm light path panel photobioreactor at constant ingoing photon flux density ($930 \mu\text{mol photons m}^{-2}\text{s}^{-1}$). At the optimal combination of biomass density and dilution rate, equal biomass yields on light energy were observed for both light paths for both microalgae. The observed biomass yield on light energy appeared to be based on a constant intrinsic biomass yield and a constant maintenance energy requirement per gram biomass. Using the model of Pirt (New Phytol 102:3–37, 1986), a biomass yield on light energy of 0.78 and 0.75 gmol photons^{-1} and a maintenance requirement of 0.0133 and 0.0068 $\text{mol photons g}^{-1}\text{h}^{-1}$ were found for *D. tertiolecta* and *C. sorokiniana*, respectively. The observed yield decreases steeply at low light supply rates, and according to this model, this is related to the increase of the amount of useable light energy diverted to biomass maintenance. With this study, we demonstrated that the observed biomass yield on light in short light path bioreactors at high biomass densities decreases because maintenance requirements are relatively high at these conditions. All our experimental data for the two strains tested could be described by the physiological models of Pirt (New Phytol 102:3–37, 1986). Consequently, for the design of a photobioreactor, we should maintain a relatively high specific light supply rate. A process with high biomass densities and high yields at high light intensities can only be obtained in short light path photobioreactors.

Keywords Microalgae · Light path · Photosynthetic yield · Photobioreactor design

Nomenclature

A	Illuminated reactor surface area (m^2)
C_x	Biomass concentration (g L^{-1})
D_0	Dilution rate; start of the D-stat (h^{-1})
D	Dilution rate (h^{-1})
d	Deceleration rate; linear D-stat (h^{-2})
d_1	Deceleration rate; proportional D-stat, proportional fraction (h^{-2})
d_2	Deceleration rate; proportional D-stat, linear fraction (h^{-2})
$m_{E,x}$	Biomass maintenance coefficient ($\text{mol photons}^{-1}\text{g}^{-1}\text{h}^{-1}$)
PAR	Photosynthetic active radiation ($\mu\text{mol photons m}^{-2}\text{s}^{-1}$, 400–700 nm)
PFD	Photon flux density ($\mu\text{mol photons}^{-1}\text{m}^{-2}\text{s}^{-1}$)
$r_{E,x}$	Specific light supply rate ($\mu\text{mol photons g}^{-1}\text{s}^{-1}$)
t	Time (h)
t_{linear}	Predefined time point at which linear part of the proportional D-stat starts (h)
μ	Growth rate (h^{-1})
V	Volume reactor content (L)
$Y_{x,E(\text{obs})}$	Observed biomass yield on light energy ($\text{g mol photons}^{-1}$)
$Y_{x,E}$	Biomass yield on light energy ($\text{g mol photons}^{-1}$)

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Introduction

Microalgae receive a lot of attention presently because of their potential to be used as a feedstock for production of biodiesel. Worldwide research programs are initiated to

develop technology for production of biodiesel from microalgae, and many new companies have been developed and most probably will be develop in the future.

Microalgae have potentially an areal productivity superior to traditional agricultural crops (Chisti 2007). Realistic estimates for areal productivity are in the order of magnitude of 40–80 t of dry matter per year depending on the technology used and the location of production (Wijffels 2008). For production of algal biomass for biodiesel purposes, it is essential that the production capacity (hectares of culture) increases and the cost of production decreases dramatically.

High volumetric productivities and high biomass yields on sunlight are needed to decrease production system volumes and to lower production costs. This can be fulfilled by cultivating the microalgae in photobioreactors with a high surface to volume ratio, i.e., a short light path, such as panel reactors. Panel photobioreactors have been demonstrated to be promising photobioreactors for the controlled cultivation of microalgae (Degen et al. 2001; Hu et al. 1998c; Hu et al. 1998b; Tredici and Zittelli 1998). Furthermore, such panels can be integrated in photobioreactors in which advanced optical engineering is used to dilute light (Gordon 2002; Zijffers et al., 2008).

Record productivities have been obtained during cultivations of the cyanobacterium *Arthrospira platensis* in short light path, 1.3 and 2.8 cm, panel reactors that were turbulently mixed (Hu et al. 1996, 1998a, b; Hu and Richmond 1996). After recalculation of the presented data, the photosynthetic efficiency showed a maximum value of around 1.5 g of biomass (dry matter) produced per mole of photons ($\text{g mol photons}^{-1}$) at incident light intensities up to $2,000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$. This high efficiency was attributed to short exposure times to (over-)saturating light intensities at the reactor surface due to turbulent mixing. The biomass yield of *A. platensis* reported by Hu et al. is close to the theoretical attainable maximum biomass yield on light energy. This can be calculated using the stoichiometric reaction equation for biomass formation on carbon dioxide, water, and nitrate or urea (Appendix).

Although the case for *A. platensis* seems to be solid, it is unclear if the outcome of this work can be extrapolated to the cultivation of eukaryotic microalgae in short light path

photobioreactors. Only a limited amount of work has been done with *Monodus*, *Chlorococcum*, *Chlorella*, and *Nannochloropsis* (Degen et al. 2001; Hu et al. 1996, 1998c; Richmond and Cheng-Wu 2001; Cuaresma et al. 2009). The photosynthetic efficiency, or biomass yield on light energy, was not explicitly presented in these studies. Based on the data given, we estimated the biomass yields for microalgae to be in the range of 0.3 to $1.0 \text{ gmol photons}^{-1}$, significantly lower than obtained with the cyanobacterium *A. platensis*.

The objective of this study was to determine the effect of biomass density and light path on the biomass yield of green algae at saturating light intensities. The maximum yield could be determined because we are able to analyze the yield as a function of biomass concentrations. For this, the algae were cultivated under pseudo steady-state conditions in D-stat cultivations: continuous cultured algae were allowed to acclimate to the light regime at a wide range of dilution rates and consequently a wide range of biomass densities (Barbosa et al. 2005; Hoekema et al. 2006). The maximal microalgal biomass yield on light energy has been determined for two different model green microalgae: the marine microalga *Dunaliella tertiolecta* and the freshwater microalga *Chlorella sorokiniana* in reactors with two light paths (1.25 and 2.15 cm).

Materials and Methods

Microalgae and Reactor Start-up

D. tertiolecta CCAP 19/6B was maintained as a suspended culture in Erlenmeyer flasks containing artificial seawater medium. *C. sorokiniana* CCAP 211/8k was maintained as a suspended culture in Erlenmeyer flasks containing M-8a medium. Prior to the D-stat experiments, the algae were cultivated batch wise in the panel photobioreactor, until a sufficient biomass density was reached to withstand the maximum incident light intensity used in the experiments. After increasing the light intensity to the maximum value, chemostat cultivation was started at 70% to 80% of the maximum growth rate. Table 1 shows the operational conditions of the different D-stat cultivations.

Table 1 Operational conditions of the different cultivations

Alga	Incident light intensity ($\mu\text{mol photons m}^{-2}\text{s}^{-1}$)	Illuminated surface area (m^2)	Light path (cm)	Aeration rate (L min^{-1})	Superficial gas velocity (m s^{-1})
<i>D. tertiolecta</i>	933	0.103	1.25	1.0	6.41×10^{-3}
	990	0.103	2.15	2.0	7.75×10^{-3}
<i>C. sorokiniana</i>	871	0.103	1.25	1.0	6.41×10^{-3}
	940	0.103	2.15	2.0	7.75×10^{-3}

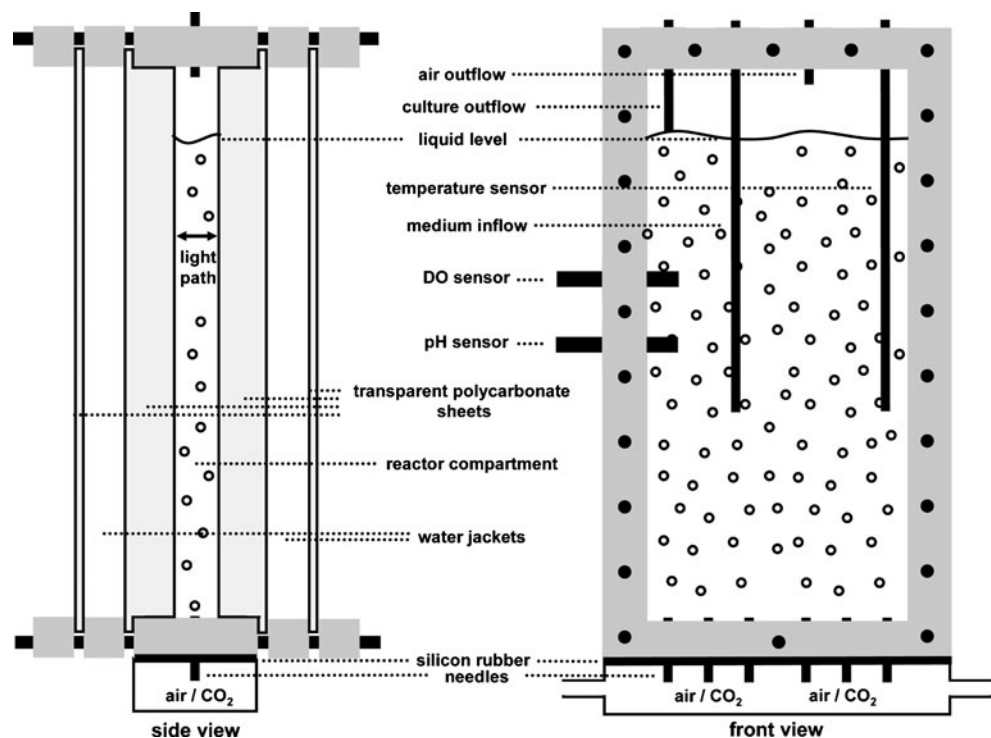
Medium

D. tertiolecta was cultivated in an enriched artificial seawater medium. The ratio between the salts, nutrient, and trace element concentrations were chosen according to the biomass composition determined by Ho et al. (2003). The *D. tertiolecta* medium was designed to support 100 g of dry weight biomass. *C. sorokiniana* was cultivated on a concentrated M-8a medium, which was based on M-8 medium developed by Mandalam and Palsson (1998). The M-8a medium was estimated to support at least 50 g of *C. sorokiniana* dry weight biomass. Sodium bicarbonate was added to both media after the pH was set. The medium used in the continuous cultivation experiments was kept under carbon dioxide atmosphere to prevent precipitation of salts. The following medium composition was used (M): *D. tertiolecta*: 0.42 NaCl, 5.6×10^{-3} MgCl₂, 3.6×10^{-4} CaCl₂, 5.3×10^{-3} Na₂SO₄, 0.10 KNO₃, 2.0×10^{-2} NaHCO₃, 6.3×10^{-3} NaH₂PO₄, 3.1×10^{-4} Na₂ EDTA, 1.1×10^{-4} FeCl₃, 6.1×10^{-6} CuSO₄, 1.4×10^{-5} ZnSO₄, 9.5×10^{-8} CoCl₂, 1.5×10^{-5} MnCl₂, and 1.0×10^{-7} Na₂MoO₄; *C. sorokiniana*: 4.8×10^{-2} CO (NH₂)₂, 1.6×10^{-2} KH₂PO₄, 4.4×10^{-3} Na₂HPO₄, 4.9×10^{-3} MgSO₄, 2.7×10^{-4} CaCl₂, 9.5×10^{-4} EDTA ferric sodium salt, 3.0×10^{-4} Na₂ EDTA, 3.0×10^{-6} H₃BO₃, 2.0×10^{-4} MnCl₂, 3.3×10^{-5} ZnSO₄, 2.2×10^{-5} CuSO₄, and 5.0×10^{-3} NaHCO₃.

Reactor Set-up and Control

Figure 1 shows a front and side view of the panel reactor. The reactor was built up by transparent polycarbonate

Fig. 1 Front and side view of the flat panel photobioreactor. The light path is determined by the thickness of the transparent polycarbonate sheets placed in the frame and was 1.25 or 2.15 cm



sheets held together in a frame similar to the system used by Barbosa et al. (2005). The panel reactor had a width of 20 cm, a height of 60 cm, and a light path as shown in Fig. 1 and Table 1.

The temperature inside the reactor was measured using a pt-100 and kept constant by an ADI 1030 Bio-controller (Applikon, Schiedam, the Netherlands) controlling the temperature of the cryostat connected to water jackets on both the front and back surface of the reactor. Temperature was maintained at 30°C and 37°C for *D. tertiolecta* and *C. sorokiniana*, respectively. The reactor content was mixed by sparging of air through needles pierced through a piece of silicon rubber at the bottom of the reactor. At least 24 needles were used to prevent cell death due to high bubble formation speeds (Barbosa et al. 2004). The airflow through the needles was controlled by mass flow controllers (Brooks Instrument, Hatfield, MA, USA). Before entering the reactor, the air was passed through a 20-l carboy with water to humidify the air to prevent evaporation of water from reactor. The pH was measured using pH gel sensors (Applisens, Schiedam, the Netherlands) connected to the ADI 1030 Bio-controller. The pH was maintained at 7.8 and 6.7 for *D. tertiolecta* and *C. sorokiniana*, respectively, by addition of carbon dioxide to the air through mass flow controllers, controlled by the ADI 1030 Bio-controller.

Illumination

The reactors were illuminated on one side using ten compact fluorescent tubes (Lynx LE 55 W, 535 mm, Sylvania,

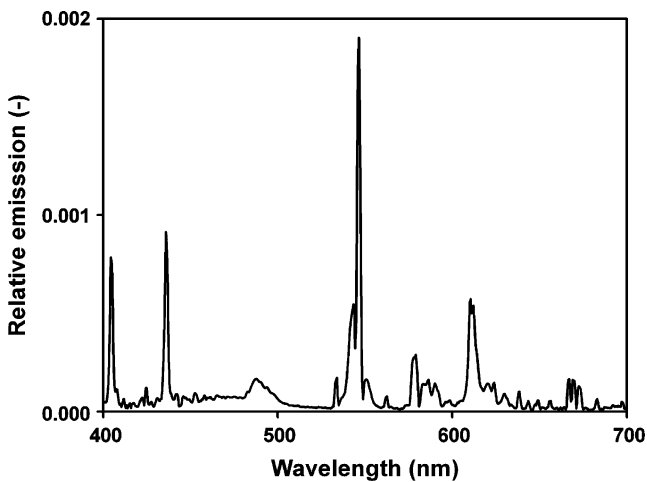


Fig. 2 Spectral composition of the fluorescent tubes after passing the first water jacket (Lynx LE 55 W, 535 mm, Sylvania, Danvers, MA, USA). The emission spectrum was normalized on a quantum basis

Danvers, MA, USA). The PAR photon flux density on the reactor surface was measured several times throughout the experiment using a Li-190sa quantum sensor (LI-COR, USA). The light absorption and reflection in passing the first water jacket was determined in an empty reactor and was corrected for in determining the photon flux density into the photobioreactor. The spectral composition (Fig. 2) of the light was determined behind the water jacket (IRRAD 2000 fiber-optic spectroradiometer, TOP sensor systems, Eerbeek, the Netherlands).

Continuous Cultivation, the A/D-stat Strategy

The yield of biomass on light energy is best investigated in chemostat cultivations where the organisms are acclimated to the conditions in the bioreactor with no biomass accumulation. In this case, the biomass yield can be directly related to a specific light availability. A disadvantage of chemostat cultures is the number of conditions that can be determined in time. Achieving steady-state conditions can take a considerable amount of time when large changes in dilution rates are made. By slowly changing the dilution rate in a photobioreactor using the A-stat method as described by Paalme et al. (1995), the microalgal productivity can be determined for a wide range of dilution rates in less time compared to performing a number of chemostat cultivations. The continuous rate of change of the dilution rate has to be chosen such that the microalgae are able to acclimate to the continuously changing conditions in the photobioreactor. In this way, the system can be considered to be in a so-called pseudo steady state, and results represent a real steady-state situation when corrected for biomass accumulation.

The flow of fresh medium into the photobioreactor was programmed and controlled through BioXpert software

(Applikon, Schiedam, the Netherlands), which determined the rotation speed of a peristaltic pump (Watson Marlow 205u, Cheltenham, UK). The actual dilution rate was determined from the decrease in weight of the medium vessel. The volume inside the reactor was kept constant by pumping excess algal suspension out through an overflow (Fig. 1).

D. tertiolecta was cultivated using a linear decrease in dilution rate using a similar rate of change as Barbosa et al. (2005). The dilution rate during the *D. tertiolecta* cultivations was calculated according to Eq. 1.

$$D = D_0 - d \times t \quad (\text{h}^{-1}) \quad (1)$$

Applying a constant deceleration rate, however, causes a rapid relative decrease in dilution rate at low dilution rates and larger deviations of the growth rate from the applied dilution rate. Because the relative change in dilution rate increases, the algae need to adapt more rapidly in order to maintain a situation representing steady state. *C. sorokiniana* was therefore cultivated using a proportional decrease in dilution rate according to Eqs. 2 and 3. Eq. 2 will never reach zero; therefore, an extra linear part was added to reach zero (Eq. 3) in the final part of the experiment. The linear part starts at t_{linear} , a predefined time at which a smooth transition to a linear decrease in dilution was made.

If $t < t_{\text{linear}}$

$$D = D_0 \cdot e^{-d_1 \cdot t} \quad (\text{h}^{-1}) \quad (2)$$

Else

$$D = D_0 \cdot e^{-d_1 \cdot t_{\text{linear}}} - d_2 \cdot (t - t_{\text{linear}}) \quad (\text{h}^{-1}) \quad (3)$$

Figure 3 shows the dilution rate development during the D-stat cultivations for the two algae. The proportional D-stat was designed to have the same duration as the linear D-stat cultivations. The dilution rate shown was determined based on the actual fresh medium supply into the reactor, measured by the decrease in weight of the medium vessel. The points shown correspond to sampling times. Using these samples, the following parameters were analyzed: dry weight biomass concentration and spectrally averaged light absorption cross section.

Dry Weight

The biomass concentration was determined by a triplicate measurement of the dry weight of biomass in a reactor sample according to Zhu and Lee (1997). Glass microfiber filters (Whatman GF/F, Kent, UK) were used to filter the microalgae. The filter weight was determined on a 0.01-mg resolution balance (Sartorius ME 235P, Goettingen, Germany). Prior to filtration, the *D. tertiolecta* sample was

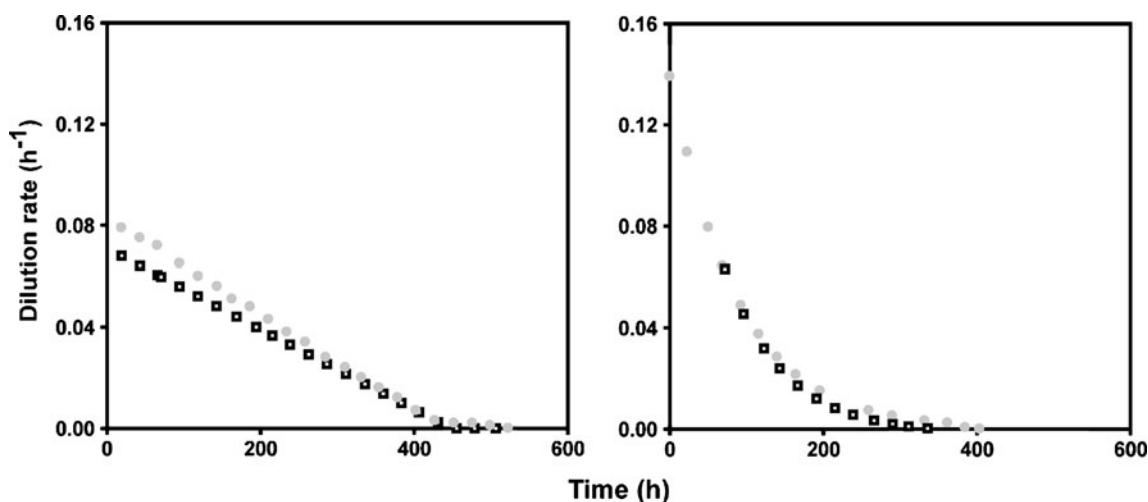


Fig. 3 Dilution rate profiles during the *Dunaliella tertiolecta* (left) and *Chlorella sorokiniana* (right) D-stat cultivations. Squares: 1.25 cm light path; circles: 2.15 cm light path

diluted ten times using a 0.5-M ammonium formate solution to dissolve any possible salt depositions in the sample. Demineralized water was used to dilute the freshwater *C. sorokiniana* sample. An additional volume of 50 ml of 0.5 M ammonium formate solution (*D. tertiolecta*) or demineralized water (*C. sorokiniana*) was filtered to further remove any small salt particles attached to the filter cake.

Absorption Cross Section

The spectrally averaged light absorption of the microalgae, a measure of the pigment content of the microalgae, was determined by measuring the wavelength-dependent light absorption of the microalgae. The absorption was measured between 400 and 750 nm using an integrating sphere (Labsphere RSA-BE-65, North Sutton, NH, USA) in a Beckman DU-640 spectrophotometer (Beckmann Coulter, Fullerton, CA, USA). The results showed residual absorption of light above 720 nm. This was attributed to backward scattering and was subtracted from the absorbance measured at all wavelengths. The wavelength-dependent specific absorption coefficient combined with the spectral composition of the radiant flux entering the reactor (Fig. 2) was used to calculate the spectrally averaged light absorption cross section according to Dubinsky et al. (1986). This spectrally averaged light absorption cross section is a measure for the specific light absorption, weighed for the light source used.

Specific Growth Rate

The specific growth rate of the organism in both the linear and the proportional D-stat cultivation was calculated from

the dilution rate corrected for biomass accumulation as shown in Eq. 4 (Barbosa et al. 2005; Hoekema et al. 2006).

$$\mu_t = \frac{\left[\left(\frac{C_{x,t} - C_{x,t+1}}{(t-t+1) \times C_{x,t}} + D_t \right) + \left(\frac{C_{x,t} - C_{x,t-1}}{(t-t-1) \times C_{x,t}} + D_t \right) \right]}{2} \quad (\text{h}^{-1}) \quad (4)$$

Observed Biomass Yield on Light Energy

The observed biomass yield on light energy was calculated by Eq. 5. The yield was based on the biomass production rate and light supply rate. The yield was not corrected for unused light passing through the flat panel photobioreactor at very low biomass densities.

$$Y_{x,E(\text{obs})} = \frac{C_x \times \mu \times V}{\text{PFD}_{\text{in}} \times A \times 3,600 \times 10^{-6}} \quad (\text{g mol photons}^{-1}) \quad (5)$$

Specific Light Supply Rate

The cultivation results were compared based on the specific light supply rate, calculated by Eq. 6. The photon flux entering the panel photobioreactor was divided by the amount of biomass present in the reactor.

$$r_{E,x} = \frac{\text{PFD}_{\text{in}} \times A}{C_x \times V} \quad (\mu\text{mol photons g}^{-1} \text{ s}^{-1}) \quad (6)$$

Results and Discussion

Biomass Concentration

The biomass concentration of *D. tertiolecta* and *C. sorokiniana* in flat panel bioreactors with light paths of

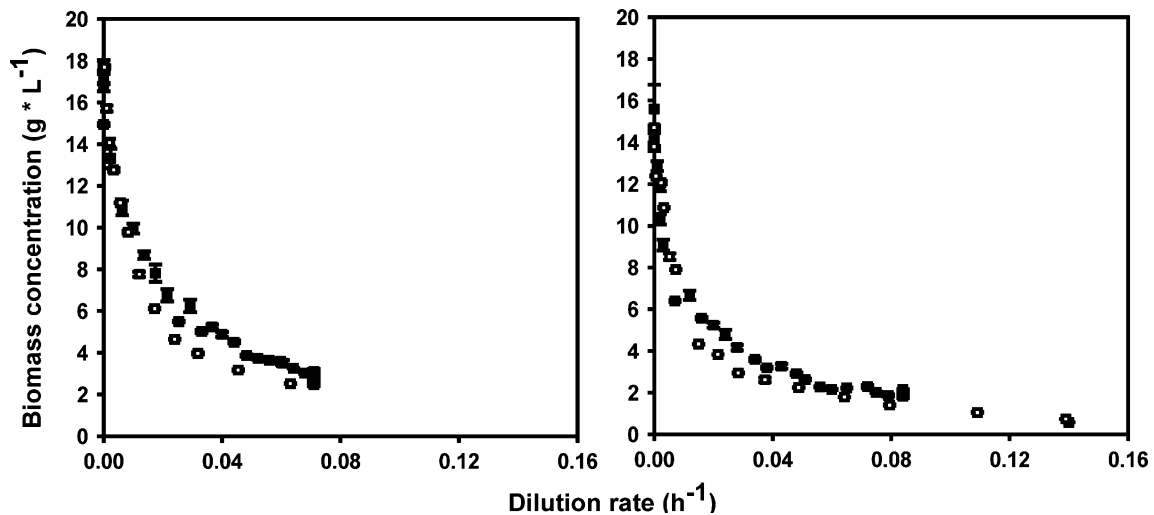


Fig. 4 Biomass concentration of *Dunaliella tertiolecta* and *Chlorella sorokiniana* during the D-stat cultivations. *Left*: 1.25 cm light path. *Right*: 2.15 cm light path. *Closed squares*: *D. tertiolecta* biomass; *open squares*: *C. sorokiniana* biomass. *Error bars* represent standard errors from the mean

1.25 and 2.15 cm as a function of the dilution rate were determined (Fig. 4). In continuous culture, the maximum biomass concentration was about 18 g/l and decreased as a function of dilution rate as expected. Higher biomass concentrations were obtained in the reactors with shorter light paths. The biomass concentrations obtained were about equal for both algae, even though the two microalgae differ in maximum growth rate and require different cultivation conditions. The two different microalgae were cultivated under controlled and optimal conditions. Concentrations of nutrients and trace elements were available in excess, pH and temperature were kept optimal, and

produced oxygen was removed by aeration. Hence, the microalgae were cultivated under light-limited conditions, and the productivity obtained at a dilution rate is a direct measure of the efficiency of use of the constant available light intensity.

Observed Biomass Yield on Light Energy

The biomass yield on light was calculated with Eq. 4 and plotted as a function of the light supply rate (the amount of light available per amount of biomass) as calculated with Eq. 5. In Figs. 5 and 6, the observed biomass yield on light

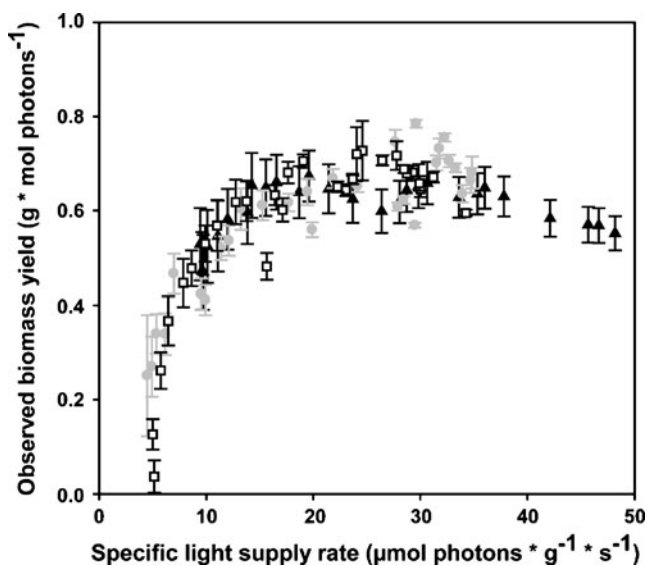


Fig. 5 Observed biomass yield on light energy during the *Dunaliella tertiolecta* cultivations. *Squares*: 1.25 cm light path; *circles*: 2.15 cm light path; *triangles*: 3 cm light path (Barbosa et al. 2005). *Error bars* represent standard errors from the mean

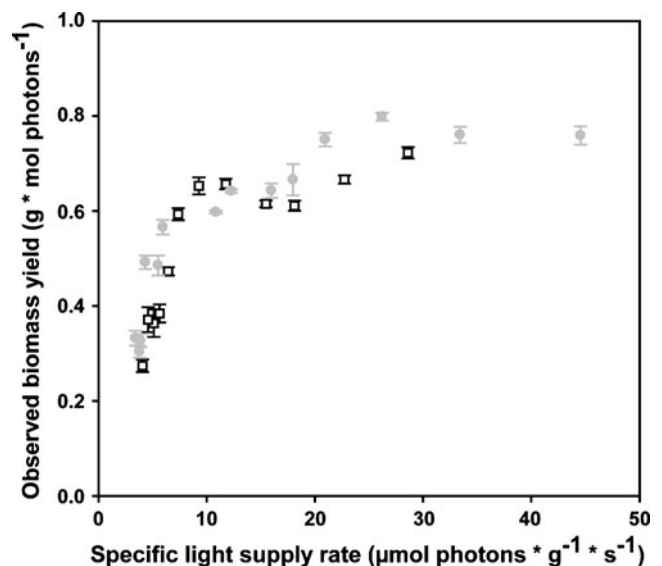


Fig. 6 Observed biomass yield on light energy during the *Chlorella sorokiniana* cultivations. *Squares*: 1.25 cm light path; *circles*: 2.15 cm light path. *Error bars* represent standard errors from the mean

energy as a function of the light supply rate at the different light paths are shown for *D. tertiolecta* and *C. sorokiniana* cultivations, respectively. In Fig. 5, we also added the data for *D. tertiolecta* in a photobioreactor with a light path of 3 cm from Barbosa et al. (2005) At higher photon flux densities, a relatively constant yield between 0.6 and 0.8 g/mol photons is obtained for all cases. At lower photon flux densities, i.e., at higher biomass concentrations, the yield drops considerably. At a given light supply rate, the highest biomass concentration is present in the 1.25-cm light path reactor and the lowest in the 3-cm light path reactor, leading to different light penetration depths in the reactors but the observed yields as a function of light supply rate in the different photobioreactors are independent of the light path used. The different biomass concentrations in the different bioreactors result in different light gradients and different exposure patterns of the algae to the light, ranging from an exposure to a steep gradient in the 1.25-cm reactor to an exposure to a more gradual gradient in the 3-cm reactor. Apparently, this gradient and the corresponding light exposure pattern does not influence the biomass yield in these reactors since the biomass yield observed at a given specific light supply rate was equal for all three reactors.

A biomass yield of 0.65 ± 0.10 g mol photons⁻¹ was obtained for *D. tertiolecta* in all panel reactors ranging from a light supply rate of 35 down to 13 $\mu\text{mol photons g}^{-1} \text{s}^{-1}$.

The yields obtained during the *C. sorokiniana* cultivations were similar to the values obtained during the *D. tertiolecta* cultivations (Fig. 6). Yields between 0.6 and 0.8 g mol photons⁻¹ were obtained between light supply rates of 45 down to 10 $\mu\text{mol photons g}^{-1} \text{s}^{-1}$.

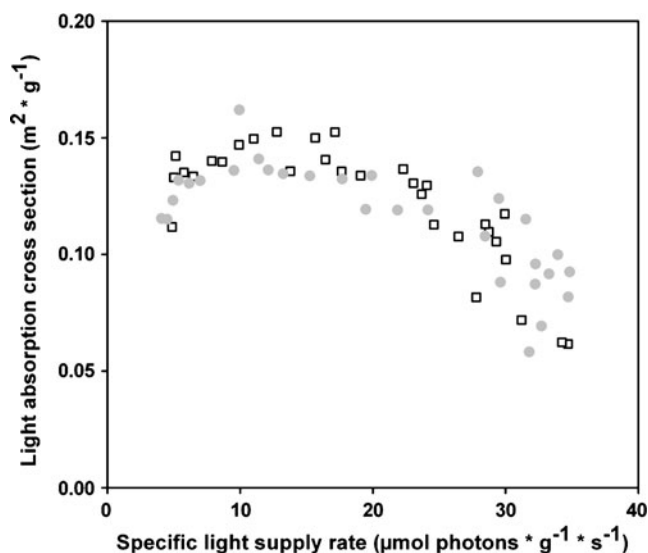


Fig. 7 Spectrally averaged light absorption cross section of *Dunaliella tertiolecta* during the cultivations. Squares: 1.25 cm light path; circles: 2.15 cm light path

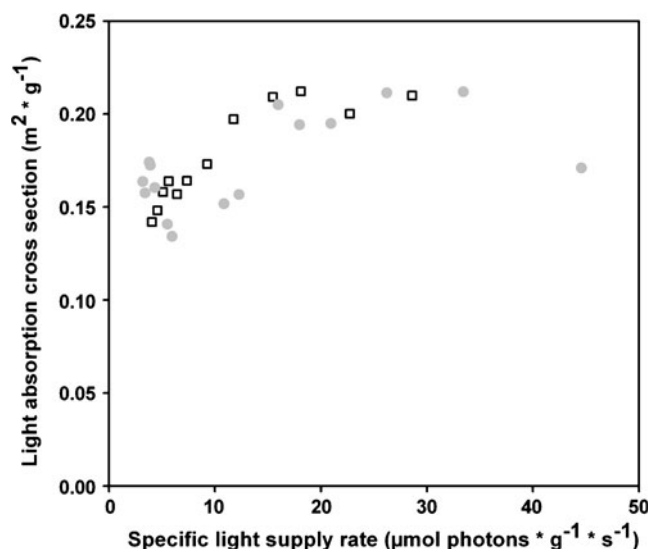


Fig. 8 Spectrally averaged light absorption cross section of *Chlorella sorokiniana* during the cultivations. Squares: 1.25 cm light path; circles: 2.15 cm light path

Absorption Cross Section

To determine the absorption characteristics at the different light supply rates, the spectrally averaged light absorption cross sections were determined as shown in Figs. 7 and 8. The light absorption cross section of *D. tertiolecta* (Fig. 7) increased with this decreasing light supply rate, indicating a photo acclimation response generally observed for microalgae subjected to decreasing light availability. The cross section increases to a maximum value until it decreases slightly at the end of the D-stat cultivation.

Figure 8 shows the light absorption cross section for *C. sorokiniana* during the two D-stat cultivations. We did not observe an increase in absorption cross section while decreasing the light supply rate. Unpublished results in a similar set-up show a smaller absorption cross section at higher light supply rates than the ones tested here. Possibly, photoacclimation of *C. sorokiniana* already occurs at higher specific light supply rates and is therefore not clearly visible in these experiments. The decrease in absorption cross section at very low specific light supply rates, on the other hand, was more pronounced during the *C. sorokiniana* cultivation. It started at a light supply rate of 20 as compared to 10 $\mu\text{mol photons g}^{-1} \text{s}^{-1}$ for *D. tertiolecta*.

Figures 7 and 8 show the microalgae adapted to the changing light supply rate by changing the absorption cross section. The decreasing light supply rate caused an increase in the light absorption cross section until the maximum size was reached. Eventually, the light supply rate was insufficient to maintain the maximum absorption cross section causing the cross section to decrease. Both the response in absorption cross section and the small difference in growth rate

compared to the dilution rate (data not shown) showed that the microalgae were able to acclimate to the changing light regime. The results obtained during the D-stat were therefore considered to be representative for a steady-state situation.

Biomass Yield on Light Energy

The biomass yield on light energy of the acclimated microalgae is determined by the ability of the algae to use the supplied light energy for biomass formation. The light energy distribution in the reactor is characterized by a high PFD on the reactor surface, which decreases rapidly due to light absorption by the microalgae, creating a photic zone and a dark zone in the reactor. The photic zone is characterized by a strong light gradient with a PFD of about $930 \mu\text{mol photons}^{-1}\text{m}^{-2}\text{s}^{-1}$ at the surface that quickly decreases to a light intensity below the compensation point at the interface with the dark zone. The compensation point is the light intensity at which the photosynthesis and respiration compensate each other, i.e., net oxygen production is zero.

Assuming a steady liquid flow, a decrease of the size of the photic zone will lead to a shorter exposure time to (over-)saturating light levels. Also, if panel width is reduced, the absolute residence time in the photic zone can be reduced, since at constant PFD, a higher biomass concentration, and thus a smaller photic zone, is obtained. Based on existing knowledge, such a reduction in exposure time to (over-)saturating light could lead to higher photosynthetic efficiencies, since less light-induced damage will build up, and possibly also, non-photochemical quenching (heat dissipation) of light energy will be less pronounced (Horton and Ruban 2005; Muller et al. 2001). Increased exposure times to (over-)saturating PFDs are

therefore expected to lead to reduced photosynthetic efficiency and vice versa.

The strong decrease of biomass yield on light energy observed at low specific light supply rates, i.e., low specific growth rates and high biomass densities, in the panel reactors, however, does not correlate to the change in light regime, i.e., the decrease in size of the photic zone. The yield decrease, on the other hand, can be explained assuming that a significant fraction of the available light energy is not used for biomass formation but it is used for basic physiological maintenance, which includes processes such as osmoregulation, cell motility, defense mechanisms, and proofreading and internal turnover of macromolecular compounds (van Bodegom 2007).

To demonstrate that maintenance requirements determined the reduction of observed yield at lower light supply rates, we fitted our data through the model of Pirt (1986), as shown by Eq. 7, for both *C. sorokiniana* and *D. tertiolecta* for the 1.25- and 2.15-cm light path panel reactors. The model assumes that the light energy available to a microalga ($r_{E,x}$; $\mu\text{mol photons g}^{-1}\text{s}^{-1}$) is either used for maintenance processes or for formation of new biomass. The model is based on a constant light energy requirement for maintenance ($m_{E,x}$; $\text{mol photons}^{-1}\text{g}^{-1}\text{h}^{-1}$). The model is also based on a constant energy requirement for the formation of biomass ($Y_{x,E}$; $\text{g mol photons}^{-1}$), which is different from the observed biomass yield on light energy shown in Figs 5 and 6.

$$r_{E,x} \times 0.0036 = \frac{\mu}{Y_{x,E}} + m_{E,x} \quad (\text{mol photons g}^{-1}\text{h}^{-1}) \quad (7)$$

During the D-stat experiment, the dilution rate through the short light path reactor decreases, and biomass accumulates. The increase in biomass concentration reduces

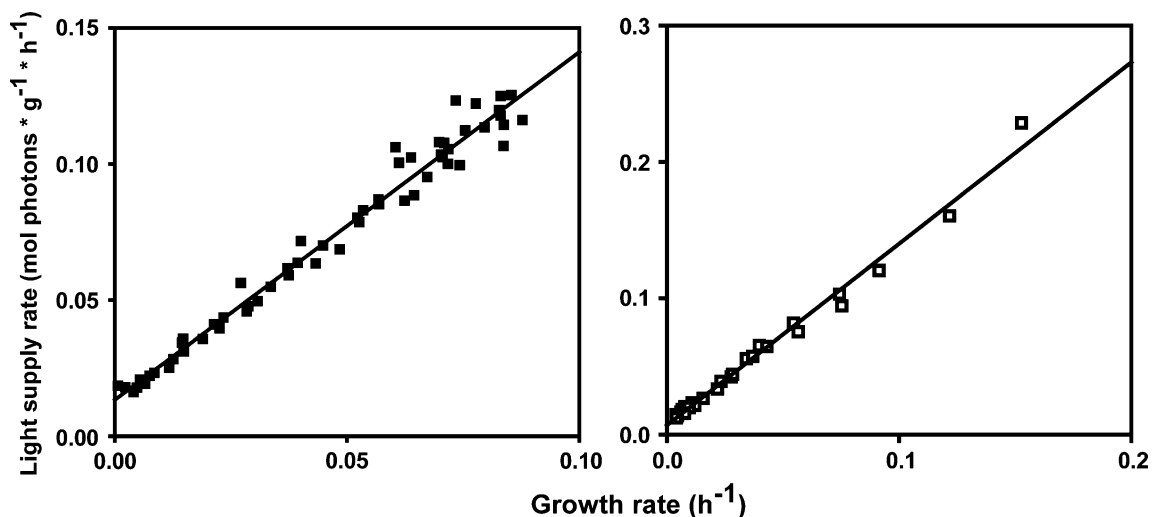


Fig. 9 Specific light supply rate in the panel reactor as a function of the growth rate. *Closed squares: Dunaliella tertiolecta; open squares: Chlorella sorokiniana*

the light energy available for the individual microalgae cells ($r_{E,x}$; $\mu\text{mol photons g}^{-1}\text{s}^{-1}$) and affects the specific growth rate (μ_t ; h^{-1}). When the light supply rate is thus plotted as a function of the obtained growth rate, a straight line should be obtained according to Eq. 7. The slope of this line represents the inverse of the yield, and the offset represents the maintenance coefficient. Figure 9 shows that the D-stat results obtained for *D. tertiolecta* and *C. sorokiniana* can be well described by a constant yield and maintenance requirement. Since no clear influence of the light path on the observed biomass yield on light energy was observed, the data for the 1.25- and 2.15-cm reactors were taken together, and the following relations were found:

$$\begin{aligned} D. \textit{tertiolecta} : \quad r_{E,x} \times 0.0036 &= \frac{\mu_t}{0.782} + 0.0133 \\ R^2 &= 0.977 (\text{mol photons g}^{-1} \text{h}^{-1}) \\ C. \textit{sorokiniana} : \quad r_{E,x} \times 0.0036 &= \frac{\mu_t}{0.751} + 0.0068 \\ R^2 &= 0.988 (\text{mol photons g}^{-1} \text{h}^{-1}) \end{aligned}$$

The constant value for the biomass yield on light energy clearly shows that the steep decrease in observed biomass yield at high biomass concentration (Figs. 5 and 6) is thus a direct result of a considerable cellular maintenance requirement. Photosynthetic activity is constant throughout the experiment, but an increasing fraction of the generated chemical energy is used for maintenance processes, decreasing net photosynthesis and biomass formation. The decrease in biomass yield commonly found at high biomass concentrations might thus not be caused by a decrease in efficiency of light use, i.e., a decrease in yield, but by an increase in maintenance energy requirement because of the increase in biomass concentration.

Cellular maintenance energy requirements of microalgae could have major implications for the development of systems for mass cultivation of microalgae. In order to optimize microalgae yield on light energy while obtaining high volumetric productivities, high growth rates need to be combined with high biomass densities. Research should thus focus on further reducing the reactor light path, such that high light supply rates can be obtained at high biomass densities. In this way, also at high biomass densities, high specific growth rates can be maintained reducing the losses associated to maintenance.

Based on the Pirt model, a constant biomass yield on light energy of 0.78 and 0.75 gmol photons^{-1} was found for *D. tertiolecta* and *C. sorokiniana*, respectively, in the current study. The yield differed from the theoretical maximum of 1.5 and 1.8 gmol photons^{-1} as calculated in the Appendix. The difference between the biomass yield and this theoretical maximum is most likely related to heat dissipation of absorbed light energy in the photosynthetic antenna complex by processes collectively called non-photochemical quenching (Muller et al. 2001). These

processes are activated when the rate of light absorption exceeds the maximal capacity of the photosynthetic machinery and prevents photodamage. In case of *D. tertiolecta*, a calculated 48% of the available light energy was not used for biomass formation, and in case of *C. sorokiniana*, it increased to 58%. Figures 5 and 6 do not directly show a difference in observed biomass yield on light energy, but the microalgae do differ in the efficiency of light use. *D. tertiolecta* has a higher maintenance energy requirement, whereas *C. sorokiniana* has a slightly lower biomass yield on light energy. The higher maintenance energy requirement causes the yield of *D. tertiolecta* to decrease faster at high biomass densities.

In literature, yield values are generally not explicitly mentioned, requiring recalculation of presented biomass production and assumptions on reactor geometry and light intensities entering the reactors and making an exact comparison of the values difficult. Our studies presented here show observed yields at different biomass concentrations and different dilution rates. The maximum observed yields that we have found are comparable to maximum yields reported in literature or which were re-calculated from literature. Meiser et al. (2004) found a maximum yield of 0.5 gmol photons^{-1} for the diatom *Phaeodactylum tricorutum* at a light intensity of 1,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Hu et al. (1998c) obtained a yield of 0.5 gmol photons^{-1} cultivating the microalga *Chlorococcum littorale* at a light intensity of 2,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Richmond et al. (2003) obtained a maximum yield of 0.6 gmol photons^{-1} cultivating the microalga *Nannochloropsis* sp. at a light intensity of 2,000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. *Monodus subterraneus* showed a maximum yield of 1.0 gmol photons^{-1} (Hu et al. 1996). This productivity was obtained at an increased level of turbulence compared to the cultivations performed in this study.

Increasing turbulence by increasing the superficial gas velocity in our cultures did not result in a clear improvement of culture productivity (data not shown). High superficial gas velocities (0.04 ms^{-1}) even lead to unstable cultures due to increased cell death and considerable foaming and, as such, biomass loss via the gas phase. High gassing rates are also not attractive for large-scale microalgae cultivation because of the large expenditures (energy and cost) associated with it.

With this study, we demonstrated that the observed biomass yield on light in short light path bioreactors at high biomass densities decreases because maintenance requirements are relatively high at these conditions. This is apparently in contradiction with the work of Richmond et al. (2003) but supported by the physiological models of Pirt (1986) and others hereafter. We demonstrated that all our experimental data for the two strains tested could be described by this model. Consequently, for the design of a

photobioreactor, we should maintain a relatively high specific light supply rate. A process with high biomass densities and high yields at high light intensities can only be obtained in short light path photobioreactors

Conclusions

The microalgae biomass yield on light energy obtained during D-stat experiments in short light path photobioreactors appeared to be determined by the specific light supply rate, which depends on biomass density, light path, and the light intensity at the reactor surface. In reactors with different light paths (1–3 cm), equal biomass yields were observed at equal specific light supply rates. This response was observed for two different species of microalgae, the marine strain *D. tertiolecta* and the freshwater strain *C. sorokiniana*. The optimal biomass density, on the other hand, was highest in the short light path reactor. The only rationale to decrease light path then is related to the requirement of high biomass concentration for more efficient processing of the biomass in industrial applications.

With this study, we demonstrated that the observed biomass yield on light in short light path bioreactors at high biomass densities decreases because maintenance requirements are relatively high at these conditions. For the design of a photobioreactor with a maximum productivity, we should maintain a relatively high specific light supply rate.

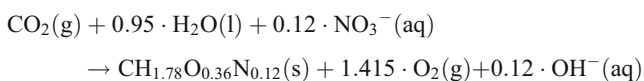
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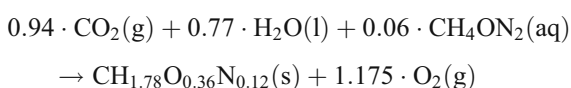
Appendix

The theoretical biomass yield on light energy can be calculated based on the stoichiometric reaction equations for the formation of biomass on carbon dioxide, water, and the nitrogen source used in the cultivation.

For growth on nitrate:



For growth on urea:



The yield of the light reactions is assumed to be 0.1 mol of oxygen per mole of photons within the PAR spectrum. This value represents the maximal quantum yield as determined under low light by several independent researchers over the past decades. The theoretical maximal based on the Z-scheme of photosynthesis would be 0.125. The difference with 0.1 is probably related to intrinsic inefficiencies of photosynthesis.

Assuming *D. tertiolecta* and *C. sorokiniana* have the same elemental composition of $\text{C}_{1.78}\text{O}_{0.36}\text{N}_{0.12}$ as found for *Chlorella Spain* sp. (Duboc et al. 1999), the molecular mass of a C-mol biomass is 21.25 g mol^{-1} . To form one C-mol of biomass, 14.15 or 11.75 mol of photons are needed to evolve the required amount of oxygen for growth on nitrate and urea, respectively, following the stoichiometric reaction equations. This leads to a theoretical biomass yield of 1.5 and 1.8 $\text{g mol photons}^{-1}$ for growth on nitrate and urea, respectively.

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