Photosynthetic efficiency of microalgae and optimization of biomass production in photobioreactors

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Hiermit wird bestätigt, dass die von Stefan Hindersin vorgelegt in englischer Sprache verfasste kumulativen Dissertation mit dem Titel „Photosynthetic efficiency of microalgae and optimization of biomass production in photobioreactors" sprachliche den wissenschaftlichen Standards genügt.

Mit freundlichen Grüßen

Prof. Myron A. Peck

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Contents

Attachment

Complete publications (Chapter 3, 4, 5)

- Influence of mixing and shear stress on *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlamydomonas reinhardtii*
- Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors
- Key parameters for outdoor biomass production of *Scenedesmus obliquus* in solar tracked photobioreactors during different seasons

Summary

This thesis focuses on specific aspects of photosynthetic efficiency of microalgae and the optimization of the biomass production in outdoor photobioreactors (PBR). The conversion of waste products from a power plant (waste heat and flue gas as CO_2 source) to microalgal biomass was investigated. If no nutrient limitation occurs the photosynthetic rate is either dominated by the temperature or light regime and the interplay between these and the culture conditions are one of the main topics of this work. It has been demonstrated how temperature affects microalgal photosynthesis and different temperature dependencies are found for specific strains (chapters 2, 5). Increased day temperatures resulted in increased productivities but are connected to high demands of waste heat. With solar tracked PBRs, the microalgal cultures are exposed to higher irradiance (up to 45%) compared to a static system, which leads to a higher biomass concentration and volumetric productivity (chapter 4 and 5). A controlled light supply in the "offset mode" enables low irradiance at low cell densities after inoculation at the initial outdoor cultivation phase (e.g. a small volume of preculture). High outdoor irradiance up to full sunlight (2000 µmol photons m^{-2} s^{-1}) can be applied without photoinhibition by adequate self shading and cell mixing (chapter 4). However, analysis of the biomass yield over 24 hours indicated light saturation for high constant PAR (chapter 5). For adequate light/dark cycles mixing is required but the resulting shear stress affects the photosynthetic activity (chapter 3). For *S. obliquus* and *C. vulgaris* optimum tip speed of 126 cm s^{-1} were found related to optimization of the PBR design. In contrast, mixing did not enhance the photosynthetic activity of the structural sensitive *C. reinhardtii*. 78 % of the yearly outdoor biomass production is generated between March and September due to the higher und longer sun radiation (chapter 5). In conclusion, the most important key parameters for process optimization are continuous adjustment of the biomass concentration and the culture temperature with respect to the fluctuating weather conditions. This optimization of the key parameter resulted in a threefold increase of biomass productivity. For modification of the cellular carbon composition, N-limitation can be used to induce carbon storage product accumulation. However, the limitation stress results in a decrease of the photosynthetic parameters (chapter 6). If primary products of photosynthesis are not used to generate new daughter cells there might be a constant biomass yield on PAR during the shift from N-sufficient growth to initial N-limitation. Finally microalgae might develop to an alternative to traditional biomass sources based on agricultural crop land. To reach this goal process optimization is essential as suggested in chapter 7 and the presented study contributes by adding new information about temperature regulation, aspects of photosynthetic efficiencies and microalgal culture operation of photobioreactors.

Zusammenfassung

Die Produktion von Mikroalgenbiomasse wird als potentielle Alternative zu erschöpflichen, erdölbasierten Rohstoffen und Energieträgern angesehen. Vor diesem Hintergrund stehen die photosynthetische Effizienz der Mikroalgen und die Optimierung der Produktionsparameter im Mittelpunkt dieser Dissertation. Die Nutzung der Abfallprodukte CO_2 (ca. 10 % des Abgases) und Abwärme eines Gaskraftwerks in die Mikroalgenkultivierung in Photobioreaktoren wurde auf Pilotmaßstab getestet. Unterliegt das Wachstum der Mikroalgen keiner Nährstofflimitierung bestimmen Licht- und Temperaturverhältnisse im Freiland die Biomassenproduktion. Es wurde die Temperaturabhängigkeit der Photosynthese einzelner Mikroalgen gezeigt (Kapitel 2 & 5). Die Erhöhung der durchschnittlichen Tagestemperatur des Kulturmediums war verbunden mit gesteigerter Produktivität jedoch ebenso mit einem hohen Bedarf an Abwärme. Zur Sonne nachgeführte Photobioreaktoren ermöglichen eine um bis zu 45 % stärkere Bestrahlung der Mikroalgenkultur verglichen mit horizontalen, statischen Anlagen. Die höhere Lichtmenge führte zu höherer Produktivität und einer höheren optimalen Zellkonzentration (Kapitel 4 & 5). Weiterhin konnte mittels Herausdrehen der Reaktoren aus direktem Sonnenlicht der Photobioreaktoren auch eine Reduktion des Lichtes im Freiland erreicht werden. Somit wurde die Photoinhibition verringert, was besonders bei niedrigen Zelldichten beispielsweise nach einem Kulturstart von Relevanz ist. Maximales Tageslicht von 2000 µmol Photonen m^{-2} s^{-1} photosynthetisch aktiver Strahlung (PAR) kann mit hoher Effizienz von Mikroalgen genutzt werden, wenn eine ausreichende Selbstverschattung der einzelnen Zellen gegeben ist. Diese wird durch starkes Mischen und der daraus resultierenden schnellen Oszillation der Zellen zwischen der lichtübersättigen und verschatteten Zonen im Photobioreaktor erreicht (Kapitel 4). Jedoch wurde eine Lichtsättigung bei hohen Tageslichtmengen (24 Stunden) für die Biomassenausbeute pro Photon festgestellt. Starkes Mischen erzeugt hohe Scherkräfte, die einerseits positiv wirken, da sie höheren Massentransfer ermöglichen, andererseits können Mikroalgen durch sie geschädigt werden. Für *Scenedesmus obliquus* und *Chlorella vulgaris* wurde eine optimale Anströmgeschwindigkeit von 126 cm s^{-1} ermittelt. Bezogen auf die photosynthetische Aktivität wurde für *Chlamydomonas reinhardtii* mit sensitiverer Zellstruktur jedoch kein positiver Effekt festgestellt (Kapitel 3). Auf Grund der Freilandbedingungen und der unterschiedlichen Lichtverteilung im Jahresverlauf ergab eine Simulation, dass 78% der Biomassenproduktion zwischen März und September generiert würde (Kapitel 5). Der wichtigste Schlüsselparameter zur Optimierung der Biomassenproduktion ist neben Licht und Temperatur die kontinuierliche Regulierung der Biomassenkonzentration in Bezug auf die fluktuierenden Lichtverhältnisse. Stickstofflimitierung wurde zur Erhöhung der kohlenstoffreichen Speicherstoffe

genutzt. Der Limitierungsstress wurde über photosynthetische Parameter erfasst (Kapitel 6). Die Ergebnisse liefern Hinweise, dass bei anfänglicher Stickstofflimitierung der Biomassenaufbau trotz geringerer Photosyntheseleistung nicht reduziert ist. Die Biomassenproduktion durch Mikroalgen sollte vertieft erforscht werden, um nachhaltige, alternative Wege der Lebensmittel-, Futtermittel- und Biotreibstoffherstellung aufzuzeigen, die bisher vorwiegend auf fruchtbarem Ackerland basiert. Optimierungsvorschläge werden in Kapitel 7 vorgestellt und diskutiert, die einen Beitrag dazu leisten dieses Ziel zu erreichen.

Chapter 1 Introduction

Solar energy

Solar radiation is the driving force for photosynthesis and the energy basis for virtually all biochemical processes of life on earth. The global net primary production of photosynthesis is estimated at 104.9 petagrams of carbon per year (Field et al. 1998). The marine microalgae net primary production is 45-50 petagrams of carbon per year (Longhurst et al. 1995). Thus, roughly equivalent amounts are contributed by land plants and marine phytoplankton communities. Also for the energy supply of mankind solar energy is used to about 90% directly or indirectly (OECD and IEA 2011). However, the greatest part based on fossil crude oil, coal, and natural gas and can by classified as non-renewable at human timescales. The conversion process from the dependency of exhaustible fossil energy towards a renewable energy supply for all human activities is obligatory for a sustainable society. In contrast to other renewable technologies biomass has the advantage that the solar energy is stored via photosynthesis and refinery processes can be applied to increase the energy density which is normally lower than traditional fossil energy carriers (Kamm and Kamm 2004).

Microalgal biomass

Biomass production by microalgae is discussed as a sustainable source of food, feed and fuel (Benemann et al. 1987; Gouveia and Oliveira 2009; Pulz and Gross 2004). For microalgal cultivation no fertile agricultural lands are necessary which is of great interest for the “food or fuel” debate about traditional land based field crops (Patil et al. 2008; Wijffels and Barbosa 2010). Different production systems like open raceway ponds and closed photobioreactors (PBR), specifically horizontal-, vertical tube systems and flat plate PBR were developed with system specific advantages. There is an ongoing discussion which system is the most promising for specific bioproducts (food, feed, fuel) (Grobbelaar 2009; Lee 2001). The optimal production system depends on the desired product and should be selected regarding different parameters, such as production costs, expenditure of work and sterility among others (Morweiser et al. 2010). Expenses are still high if biomass production is restricted to small scale devices but these costs might be reduced when production occurs on a large industrial scale (Norsker et al. 2011). Certainly, all production routes require a suitable combination of PBR design, cultivation and processing technology (Posten 2009).

Possible products from microalgae are health- and functional food, feed additives for aquaculture, biogas, biohydrogen or biofuels. Microalgal biomass is already used to produce vitamins, coloring substances, antioxidants, polyunsaturated fatty acids, enzymes such as superoxide dismutase or restriction enzymes and polymers such as special polysaccharides (Benemann et al. 1987; Cohen 1999; Pulz and Gross 2004; Pulz et al. 2008; Skulberg 2000). Microalgal cultivation for production of energy carriers like biofuels, biogas and biohydrogen requires optimization of the overall energy balance to reach an economically positive process (Hulatt and Thomas 2011; Morweiser et al. 2010; Pulz and Gross 2004; Skulberg 2000). The evaluation of the optimum culture conditions at outdoor up scaled systems for the development of microalgal technologies is still ongoing (Grobbelaar 2012). However, today only high value products from microalgae are economically successful. Therefore the general aim is to identify the key parameters that are governing the cultivation process. This knowledge of the dependency of biomass production upon various growth factors will lower the product price improving the overall economics (Norsker et al. 2011).

For outdoor cultivation at higher latitudes, open ponds are difficult to operate, because of the unfavorable, variable climatic conditions, i.e. rain and a large temperature range. Thus, closed systems are needed for such applications (Borowitzka 1999). Closed PBRs face other specific problems compared to open ponds, like overheating, supersaturation of oxygen and high energy demand for mixing (Grobbelaar 2009). Advanced productions systems have higher investment costs for infrastructure than open race way ponds which results in increased prices per kg CDW. A study comparing open ponds, horizontal tubular and PBRs system suggested prices of 4.95 , 4.15 and 5.96 € kg^{-1} CDW, respectively for a 100 ha plant simulation (Norsker et al. 2011). Despite the highest production cost in PBRs there is also the greatest potential of price reductions. By optimization of the process parameter the prices might be lowered to 0.68 € kg^{-1} CDW for flat panel PBRs (Norsker et al. 2011).

Photosynthesis of microalgae in outdoor photobioreactors

During natural diurnal cycles periods of high solar irradiances occur that are generally above the light saturation of photosynthesis (Suh and Lee 2003). This can cause photoinhibition of photosynthesis (Vonshak and Guy 1992) and decreases energy utilization of photosynthesis for biomass production in microalgal cultures (Cuaresma et al. 2011). This occurs especially during cultivation at low cell densities where less self-shading does not prevent photoinhibition. The decrease of irradiance by self shading prevents photoinhibition. Longer periods of strong light stress

may even cause photo-bleaching of cells, resulting in a collapse of the culture (Carvalho et al. 2011). The light saturation effect can be described with parameter of a rapid light curve (RLC) (fig. 1).

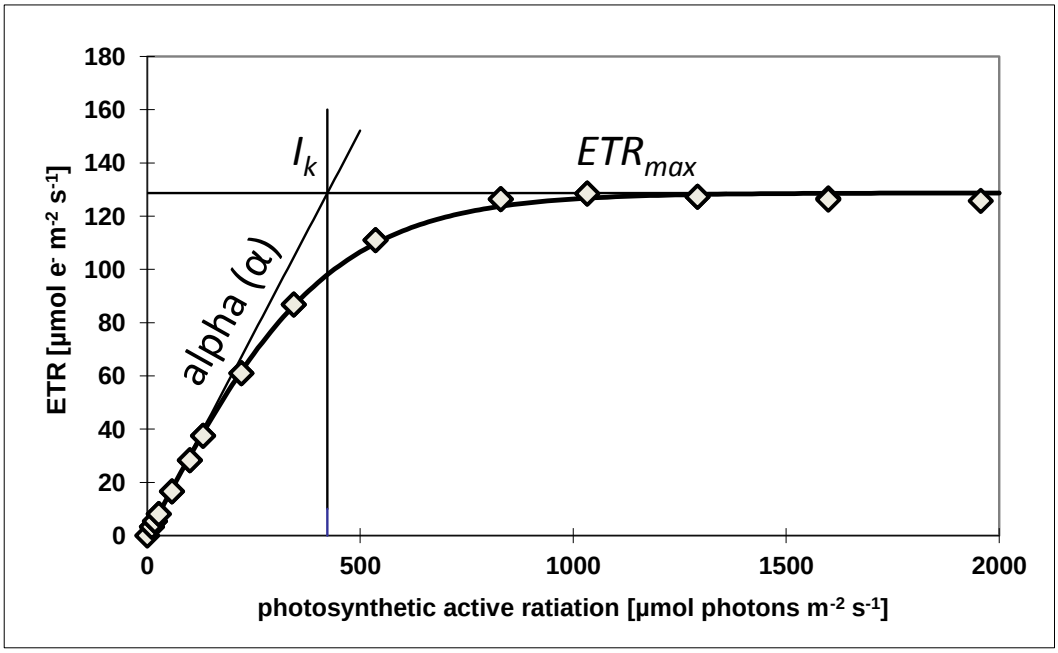

Fig. 1 Typical rapid light curve (RLC) for a microalgal culture. Photosynthetic rate is measured as the electron transport rate (ETR) of the photosystem I and II (PS (I) and PS (II)). The linear increase of the photosynthetic rate is given by slope alpha (α) at light limited irradiances. Light saturation of photosynthesis is characterized by the maximum *ETR* of PS I or PSI ($ETR_{max}(I)$, $ETR_{max}(II)$) which represent the photosynthetic capacity. The saturating irradiance I_k (I_k = (maximum photosynthetic rate)/α) represents the intersection of the slope alpha (photosynthetic efficiency) and the maximum *ETR*.

Chlorella spp. and *Scenedesmus spp.* are widely used microalgae for outdoor mass cultivation (Doucha et al. 2005; Hulatt and Thomas 2011). Both species have high growth rates in comparison the other microalgae and mass cultures can be adapted to high irradiance of direct sunlight (resulting in high ETR_{max}). Thus, these species were used for experiments of mass cultures in photobioreactors focusing on optimizing the photosynthetic efficiency (PE). A high alpha (α) of the single cells in the mass cultures indicates an efficient conversion of photon energy into primary products. This can be reached by acclimation to low light due to high self shading even under outdoor conditions.

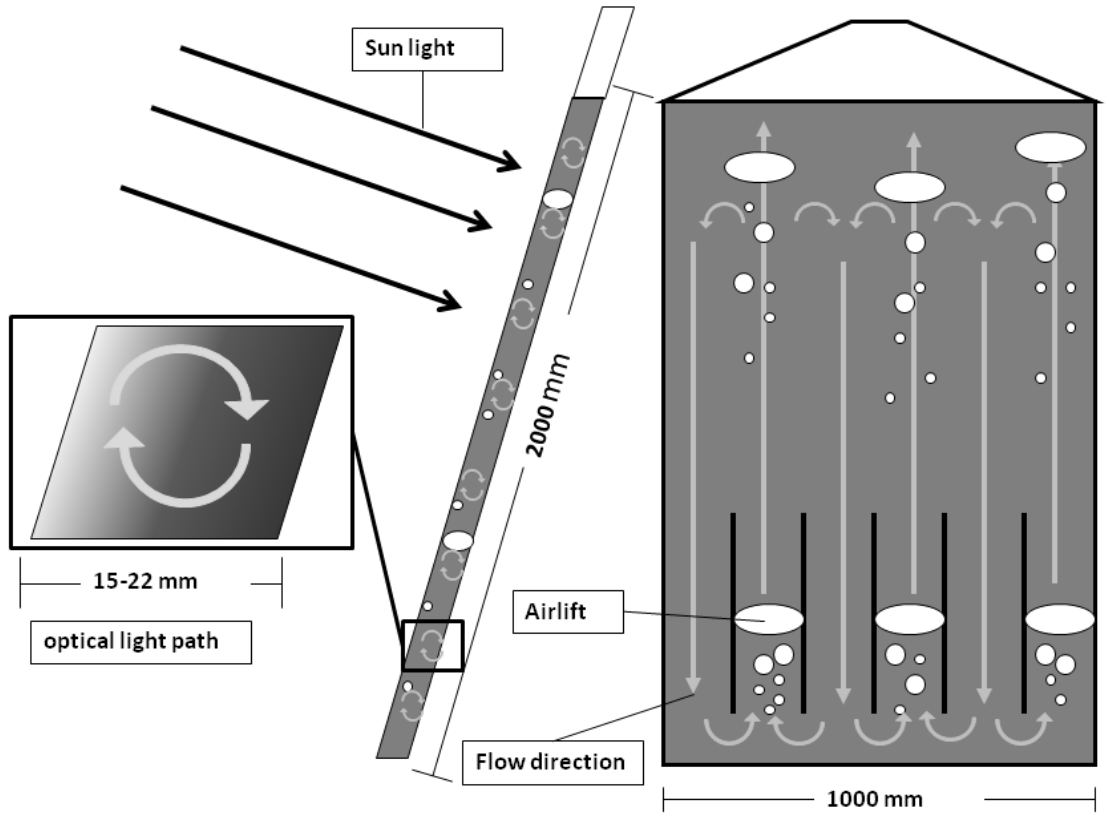

Fig. 2 Flat panel photobioreactor used in the present study. Optical light path and self shading effect indicated by gray intensities (left); photobioreator from the side (center); photobioreactor front view with flow direction of the airlift mixing (right).

The absorption of light (photosynthetic active spectrum, 400 to 700 nm) in microalgal cultures can be described by the Lambert–Beer law ($PAR = PAR_0 * exp^{-L*K_a*CDW}$) where the photosynthetic active radiation (*PAR*) is the irradiance of interest, PAR_0 is the incident PAR, *L* is the light path in [cm] and K_a is the light absorption coefficient [m^{-1}] of the specific microalgal culture and the biomass concentration (cell dry weight (CDW)) of the culture [g L^{-1}]).

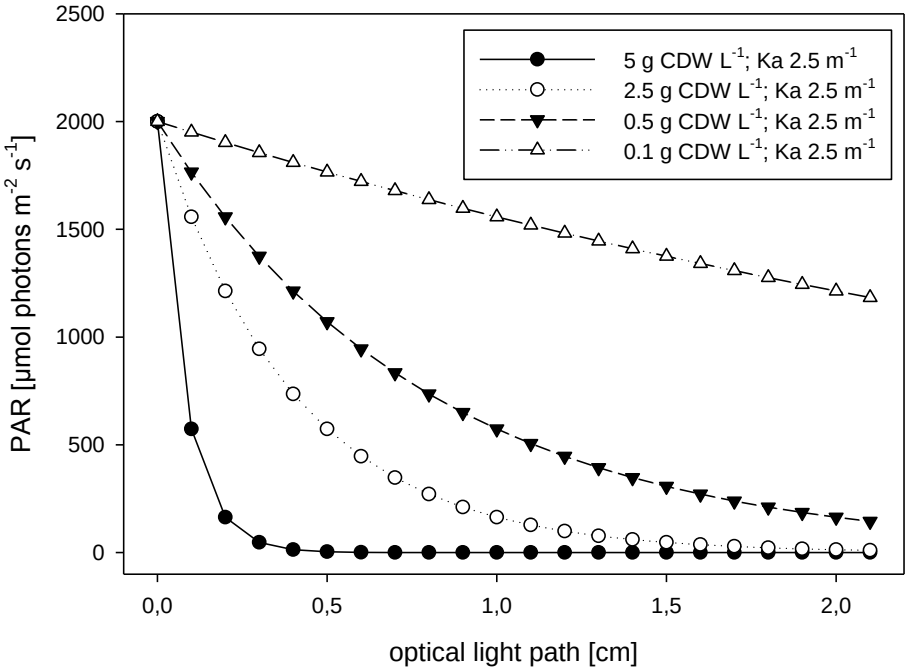

Fig. 3 The absorption of the photosynthetic active radiation (PAR) by a *Scenedesmus obliquus* culture at various biomass concentrations in dependency of the optical light path in the photobioreactor.

Strong mixing in combination with a short light path results in an intermittent light regime of short light/dark cycles to which the microalgal cells are exposed between surface and deep layers, especially when cell densities are high. However, hydrodynamic forces always have a positive effect on mass transfer and overcome boundary layers around the cell and they have a negative effect due to morphological stress. A mixed, high density cell culture can be exposed to higher irradiance above the I_k values of a single cell without causing photoinhibition due to the self-shading effect (Grobbelaar 2010; Kliphuis et al. 2010; Zou and Richmond 1999).

Specific aims of this dissertation

For phototrophic growth inorganic macro- and micronutrients, CO_2 and H_2O are needed as the elementary basis. To maximize the biomass productivity of the microalgae all growth parameter should be close to the optimum for the specific microalgae strain. This thesis focuses on specific aspects of photosynthetic efficiency of microalgae and the optimization of the biomass production in outdoor photobioreactors. If no nutrient limitation occurs the photosynthetic rate is either dominated by the temperature or light regime and the interplay between these and the culture conditions are one of the main topics of this work.

According to chapter 2

In this research study the dominant fast growing *Scenedesmus obliquus* was isolated from a non aseptic mass culture. The strain revealed to be the outcompeting microalgae for the tested culture conditions with flue gas as the only CO_2 source. Therefore the first aim was to determine the optimum temperature for photosynthesis of this strain to optimize conditions for all consecutive cultivation experiments.

Under outdoor conditions adjustment of the culture temperature in photobioreactors is technically and economically demanding. Often microalgae are exposed to suboptimal temperature at high irradiance. The second aim was to study the effect of these conditions upon photo acclimation using controlled lab experiments with a cryophilic *Chlorella* sp. species and the mesophilic *Chlorella vulgaris*.

The regulation of the temperature regime is one of the key parameter for process optimization of photobioreactors. The third aim of this study was to evaluate the heat energy fluxes at a photobioreactor pilot plant for different prototypes of photobioreactors and different weather conditions which are valuable information for the biosystem engineering part of up scaling.

According to chapter 3

Outdoors cultures are exposed to high irradiance of photosynthetic active radiation (PAR) up to 2000 µmol photons m^{-2} s^{-1}. High biomass production at maximum irradiance is possible if the average PAR intensity per irradiated microalgae cell is lowered to light limited levels. Prerequisite is sufficient self shading by high biomass concentration and an oscillation of the cells between the high PAR layer and the shaded low PAR layer within the photobioreactor. Fast oscillation is realized by the air lift principle where a gas (air or flue gas) is injected at the bottom of the photobioreactor. However, mixing of the culture causes shear stress and different microalgae species reacts differently to certain levels of shear stress. Thus, short term laboratory experiments were performed in a stirring tank (microcosm) to study the effect of mixing and shear stress on the photosynthetic activity of *Chlorella vulgaris*, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* via pulse amplitude modulation (PAM) fluorometry.

According to chapter 4

If non elementary mass limitation occurs and the optimum temperature is adjusted, the light regime remains as the main factor dominating the productivity. Different angles of flat panel PBRs to the position of the sun can be adjusted using solar trackers which are commercial available for

photovoltaic panels. Applying this technology to microalgal cultivation irradiance optimization was realized for different biomass concentrations by either an irradiance reduction if photoinhibition occurred or irradiance increase to increase the biomass productivity during light limitation.

According to chapter 5

The overall aim of this thesis was the optimization of the conversion of waste products from a power plant (waste heat and flue gas) to valuable microalgal biomass. Therefore, *Scenedesmus obliquus* was cultivated under non aseptical conditions in solar tracked photobioreactors in experimental phases over two years. The specific aim was to evaluate the key parameter for process optimization and to develop a prediction model for the biomass productivity depending on the available solar energy within the year.

According to chapter 6

For the modification of the cellular composition N-limitation can be used to induce accumulation of carbon storage products like of starch and lipids (Dragone et al. 2011; Harrison and Griffiths 2009). However, the limitation stress results in a decrease of the growth rate. Thus, the starch or lipid contents are negatively correlated to the growth rates of the microalgae (Williams and Laurens 2010). If photosynthesis do not contribute to the generation of new cells and only produce carbon storage products there might be a constant biomass yield on PAR during the shift from N-sufficient growth to initial N-limitation (Brányiková et al. 2011). The final aim was to determine the photosynthetic performance and the biomass yield on PAR (calculated from the formation of biomass per quanta of PAR photons) during outdoor N-limitation in PBR experiments.

Chapter 2 Temperature dependency of microalgal photosynthesis

Introduction

For all the parameter photosynthesis, productivity, growth- and respiration rate, there are typical temperature characteristics (minimum, optimum and maximum). These also differ with the composition of the culture medium and the irradiance regime and they are species-specific. To maximize the outdoor microalgal biomass production two principal strategies are possible: Firstly, to screen for microalgae which perform superior under the given temperature conditions or secondly to adjust the culture temperature to optimum levels of the microalgae of interest. Here, the temperature dependency is characterized for photosynthesis of a newly isolated *S. obliquus* strain. Subsequently, the growth and photosynthetic characteristics at low temperature (winter simulation) were studied for a cryophilic and mesophilic *Chlorella sp.* and the energetic effort of outdoor temperature regulation was evaluated. The cultivation of cryophilic microalgae in winter might reduce the demand of waste heat from power plants.

Temperature optimum of photosynthesis for *Scenedesmus obliquus*

Materials and methods

Scenedesmus obliquus was adapted to a PAR intensity of 200 µmol photons m^{-2} s^{-1} at a biomass concentration of 1 g CDW L^{-1} and non limiting inorganic salt concentrations. The temperature of the culture was increased stepwise by 5 °C from 15 °C to 45 °C. Each temperature level was kept constant for 3 hours to allow temperature acclimation. Photosynthetic activity of the microalgal cultures was measured using pulse amplitude modulation (PAM) fluorometry (Dual PAM 100, Walz, Germany). The maximum quantum yield (F_v/F_m) of photosystem II (PS II) was measured after 5 min of dark acclimation in a photometer cuvette using 3 mL of outdoor culture, at the constant given temperature with a maximum saturation pulse of about 20000 µmol photons m^{-2} s^{-1} and a pulse length of 300 ms. To investigate the dependency of the photosynthetic rate on the light intensity rapid light curves (RLCs) were performed and the maximum electron transport rate (ETR_{max}), alpha (α) and the characteristic light saturating intensity (I_k(II)) were calculated. For calculation of *ETR* the following calculation was used: *ETR* = PAR x standard absorption factor (AF=0.84) x 0.5. However, AF was not determined and could have been different from standard AF (Beer and Björk 2000; Soni et al. 2012; Wagner et al. 2006). Thus, to convert the *ETR* values into relative *ETR* (r*ETR*) the factor of 2,38

can be used. Each light step during the RLCs was 30 s and 12 intensities within a range from 0 to 2000 PAR µmol photons m^{-2} s^{-1} were used. Using a cryostat the temperature in the measuring cuvette was kept constant and at the same level as in the experimental culture.

Results

In the temperature range from 15°C to 45°C the photosynthetic activity of *Scenedesmus obliquus* was highest at 30°C for the maximum electron transport rate (ETR_{max}(II)) and for the minimum saturating Intensity (I_k(II))(Fig. 4). From 15°C to 30°C there was a 6 fold increase in the ETR_{max}(II) while the maximum quantum yield of photosystem (II) (F_v/F_m) stayed relatively constant (0.82 to 0.73) from 15°C to 40°C and decreased from 0.73 to 0.40 in the 40°C and 45°C treatment, respectively.

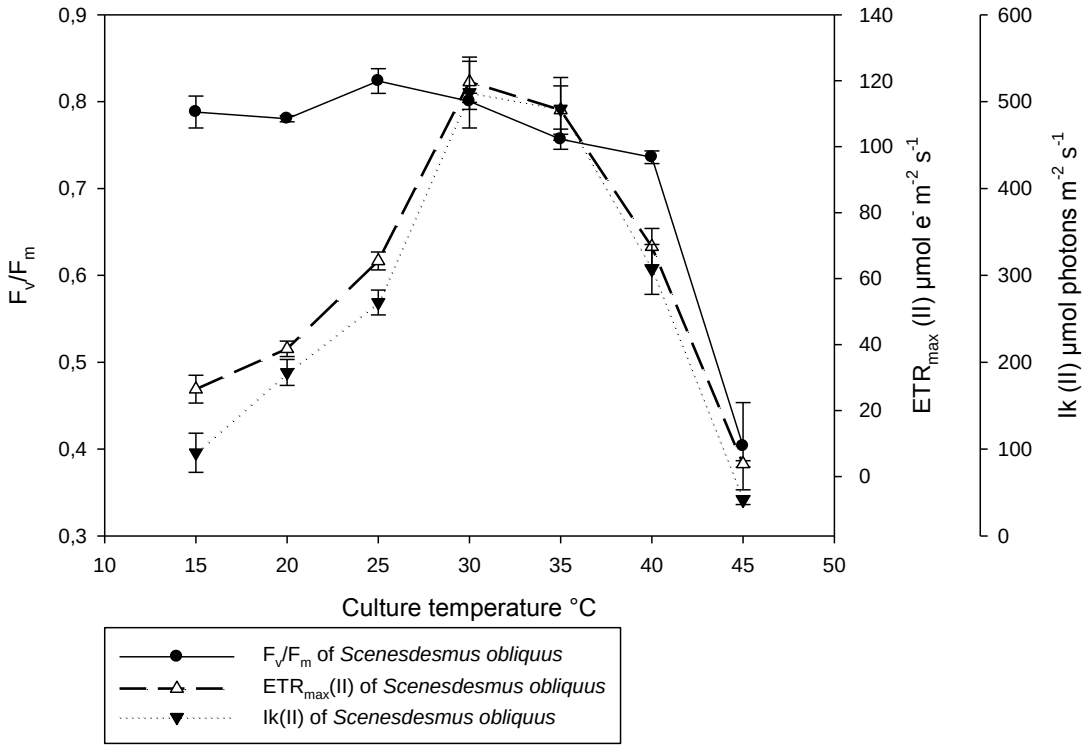

Fig. 4 The photosynthetic parameters of the photosystem (II) of *Scenedesmus obliquus* in dependence of the culture temperature.

Low temperature cultivation and photoinhibition at suboptimal temperatures of *Chlorella vulgaris* and cryophilic *Chlorella sp. "CCCryo"*

Materials and methods

The microalga *Chlorella vulgaris* (Chlorophyta) (No. 10162) was obtained from the SVCK algal collection at the University of Hamburg. The cryophilic *Chlorella sp.* "CCCryo" (No. 297-06) was provided from the CCCryo culture collection at the Frauenhofer IBMT. Both strains were cultured under unialgal conditions in a culture medium containing 2 g L^{-1} ''Flory Basic Fertilizer 1'' (Euflor, Germany) and 3.22 g L^{-1} KNO_3 in bubble columns with a working volume of 350 ml. The cultures were bubbled with filtered air and the pH-value was kept below 7.0 by injecting pure CO_2. The tanks were irradiated 24 h at a constant PAR intensity of about 200 µmol photons m^{-2} s^{-1}. The batch cultivation was used to determine the maximum growth rate (exponential phase) for the strains at 5°C.

To determine the effect of low temperatures and high irradiance the microalgae were exposed to medium light stress (1300 to 1400 µmol photons m^{-2} s^{-1}) and afterwards to high light stress (2000 µmol photons m^{-2} s^{-1}) under the culture conditions described above. Equal self shading was reached by adjustment of the cell concentration to 10^8 cells ml^{-1}. This was done using a cell counter (Beckmann Coulter counter, series Z2, Germany). The adjusted cell concentration corresponded to a cell dry weight (CDW) concentration of about 1 g L^{-1}. Directly after the application of light stresses to the cultures the photosynthetic parameter were determined via RLC and PAM methods described above.

Results and discussion

The maximum specific growth rate during the exponential growth phase at 5°C (±2°C) was 0.44 $\mu_{max5°C}$ d^{-1} and 0.79 $\mu_{max5°C}$ d^{-1} for *C. vulgaris* and the cryophilic *C.* sp. "CCCcryo"., respectively. Thus, the minimum doubling time for *C. vulgaris* was 1.5 days and 0.89 days for *C.* sp.. These results revealed that cryophilic microalgae might be used for outdoor winter cultivation in photobioreactors. However, in winter with clear weather, irradiance levels above the light saturation still occur. Therefore the effect of light stress under low temperature was determined by laboratory experiments.

Medium light stress

For medium light stress levels of PAR irradiance of 1300 - 1400 µmol photons m^{-2} s^{-1} the ETR_{max} of the PS (II) (ETR_{max} (II)) decreased from 60 [µmol electrons(e^-) m^{-2} s^{-1}] and 40 [µmol e- m^{-2} s^{-1}] to 40 [µmol e- m^{-2} s^{-1}] and 30 [µmol e- m^{-2} s^{-1}] within 3 hours for *C.*sp. "CCCcryo" and *C. vulgaris*, respectivly (Fig. 5)**.** Thus, both species decreased their photosynthetic capacity of PS (II). At low light acclimation state before medium light stress the activity of PS (I) and PS (II) were at the same level (Fig. 5 A and D). However, the medium light stress resulted in increased activty in PS (I) over time for both species. This difference between the two photosystems can be explained by an increase in the cyclic electron transtport rate and the findings are in agreement with the literatures (Joliot and Joliot 2006).

Normally, to fuel the Benson-Calvin cycle a ratio of 3:2 of ATP and NADPH is required, but the ratio is not completely satisfied by the production of ATP and NADPH via liniear electron transport mechanisms (Eberhard et al. 2008). This discrepancy is compensated by the cyclic electron transport (Johnson 2005; Joliot and Joliot 2006). It was reported that the contribution of the cyclic electron transport rate ranges from 0 to ∼50% for weak to saturating light, respectively (Joliot and Joliot 2006). These findings are in agreement with the present results were the *ETR* (I) was rougly doupled in comparison to *ETR* (II) under light stress conditions (Fig.5 B,C, E,F)(Yamori et al. 2011). Furthermore, it is suggested that the ATP concentration is the parameter which controls the activity of PS (I) and PS (II). Here, light stress at low temperatures migh induce a higher demand of ATP with respect to the NADPH because ATP is metabolized by repair mechanisims and by alternative ATP sinks than in the Benson-Calvin cycle (Eberhard et al. 2008).

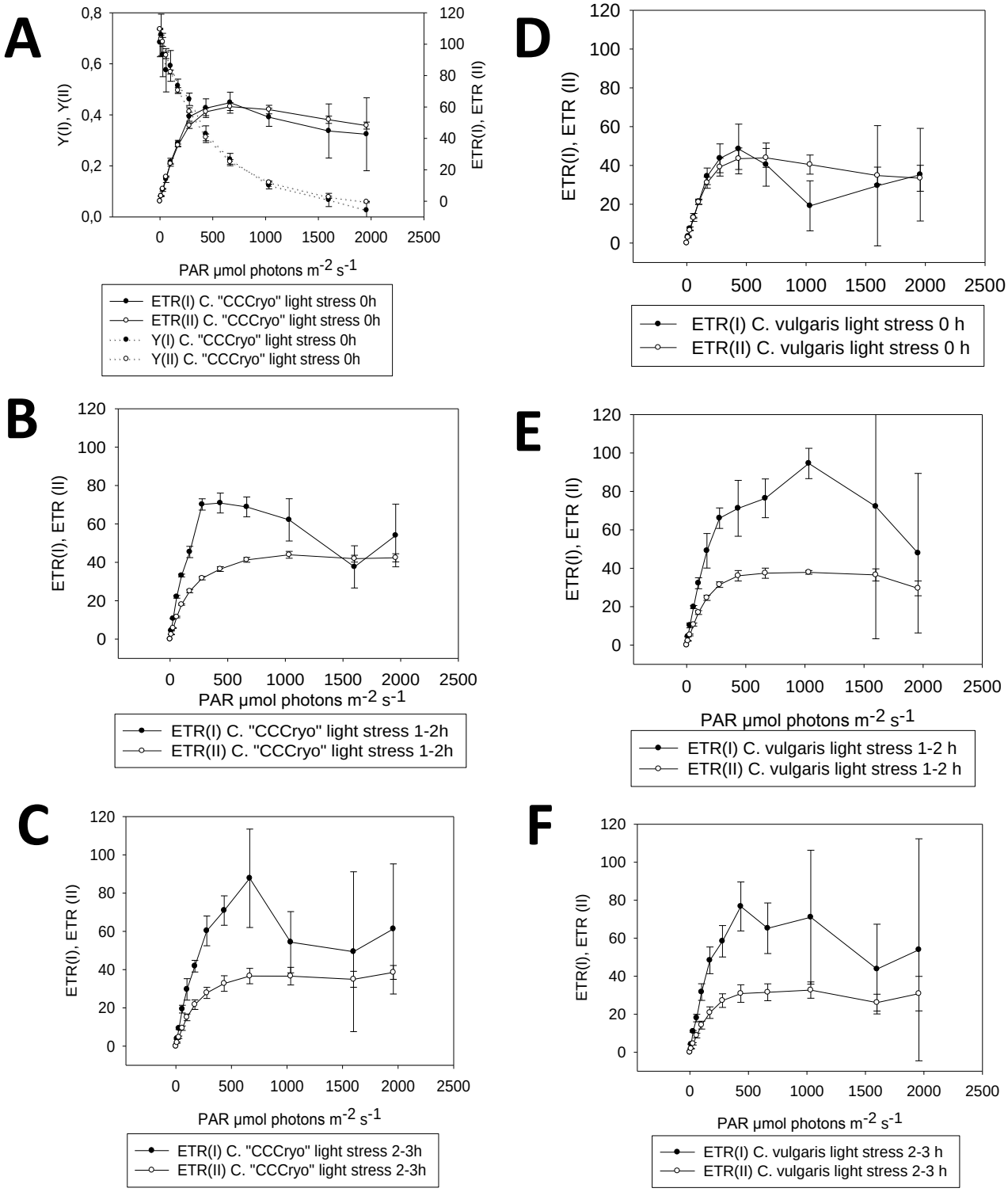

Fig. 5 The electron transport rate (*ETR*) [µmol e^- m^{-2} s^{-1}] of PS (I) and PS (II) of the cryophilic *Chlorella sp.* "CCCryo" (A-C) and the mesophilic *Chlorella vulgaris* (D-F). *ETR* was determined via rapid light curves after medium light stress of 1300 - 1400 µmol photons m^{-2} s^{-1} for different durations (0h, 1-2h, 2-3h) under low (5°C ±2°C) culture temperature conditions.

High light stress

To determine the stress level at which the activity of the PS (I) is lowered, the light stress was increased to high light stress at PAR 2000 µmol photons m^{-2} s^{-1} for the duration of 5 hours. At this irradiance both the ETR_{max} (I) and the ETR_{max} (II) of *C. sp.* "CCCryo" and the *C. vulgaris* decreased (Fig. 6 A). At the start of the experiment the ETR_{max} (I) and ETR_{max} (II) were 48 µmol e- m^{-2} s^{-1} and 38 µmol e- m^{-2} s^{-1} for *C. sp.* "CCCryo" and 20 µmol e- m^{-2} s^{-1} and 18 [µmol e- m^{-2} s^{-1}] for *C. vulgaris,* respectively. During high light exposure ETR_{max} (I) and ETR_{max} (II) decreased nearly linearly in both species. Also, same decreasing trends were detected for alpha (II). Interestingly, alpha (I) stayed relatively constant over the time of the experiment. This might indicate that the cells have a specific capacity for the ATP turnover in their metabolism because alpha is evaluated in the light limited region of the RLC (Avenson et al. 2005). Consequently, I_k (I) (effected by alpha and ETR_{max}) decreased while I_k (II) of *C. sp.* "CCCryo" remained stable or increased for *C. vulgaris*.

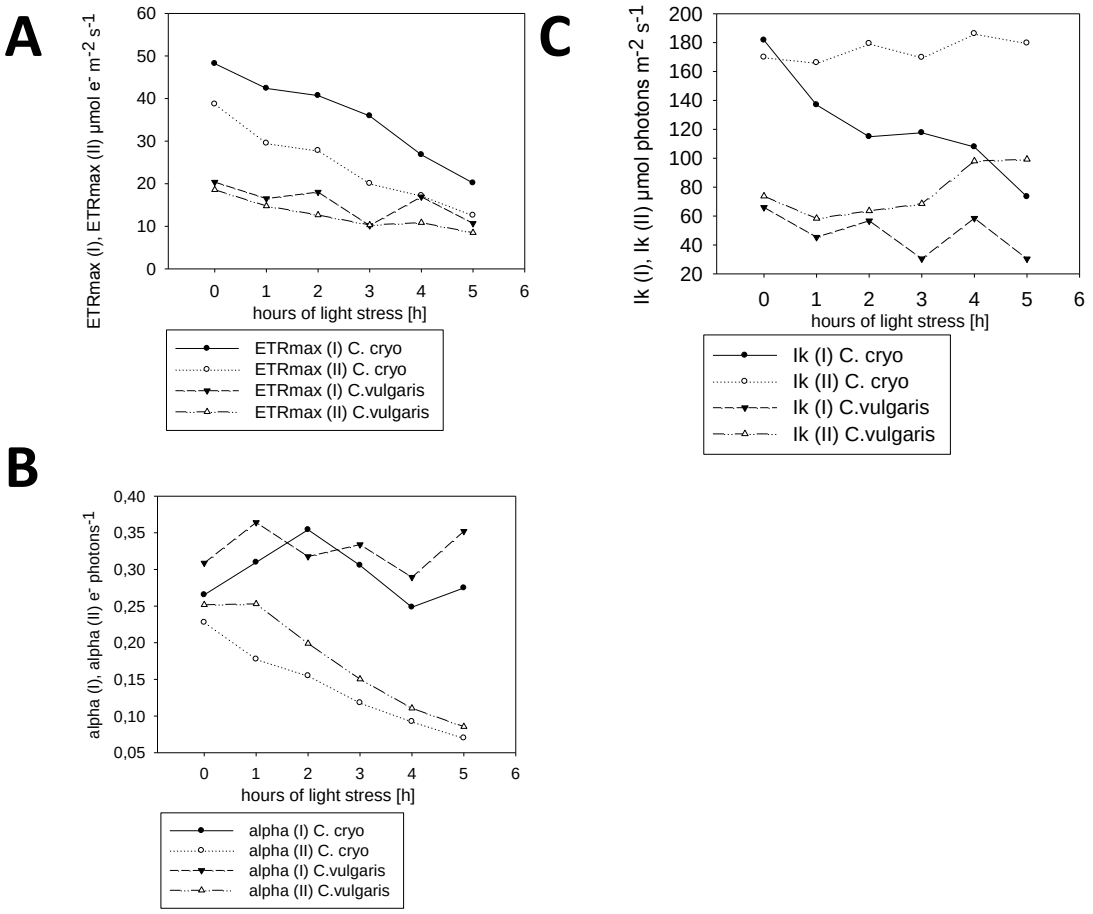

Fig. 6 Photosynthetic parameter of rapid light curves (*ETR* (A), alpha (B), I_k(C)) of the cryophilic *Chlorella sp.* "CCCryo" and the mesophilic *Chlorella vulgaris* during high light stress (PAR: 2000 µmol photons m^{-2} s^{-1}) under low (5°C ±2°C) culture temperature conditions.

In comparison to medium light stress the activity of PS (I) decreased at suboptimal temperatures and under high light conditions. It can be suggested that under these conditions the cellular metabolism was limiting the demand of ATP resulting in the synchronic downregulation of PS (I) and PS (II) activity. Under these conditions the extra energy supply to the photosynthetic apparatus cannot be used because the enzymatic reaction of the Benson-Calvin cycle might be temperature limited. Hence, it is concluded that the irradiance level per cell is responsible for either an increased or stable ratio of *ETR* (I)/*ETR* (II) during light stress conditions.

These results demonstrated the superior photosynthetic activity of the cryophilic *Chlorella* sp. in comparison to the mesophilic *Chlorella vulgaris* under suboptimal temperatures and high irradiance.

Energetic effort for outdoor temperature regulation

The photoinhibitory effects of high irradiances and low temperatures have been demonstrated (medium and high light stress). Also temperatures above the optimum decreased the photosynthetic performance (Fig. 4). Thus, for optimization of outdoor production the culture temperatures were regulated as described in detail in Chapter 4 and 5. Also the flow scheme of the pilot plant and the heat exchangers are presented in chapter 4. However, due to outdoor conditions the temperature inside the PBR were kept within a wider range from 10°C during frost periods to 35°C during summer using active cooling. The influence of air temperature and PAR on the culture temperature for a winter and summer situation are shown in Fig. 7 including the heat demand and heat supply, respectively. High PAR of 1500 µmol photons m^{-2} s^{-1} drastically reduced the heat requirement in winter even if the air temperature was below 0°C (Fig. 7 day 26.12.2010). This is based on the efficient light absorption (Fig. 3) by the microalgae culture, which resulted in heating of the culture.

Tab. 1 The heat demand during a typical frost situation in winter (6 days of cultivation in Dec.) and the heat surplus which has to be removed during hot summer conditions (5 days of cultivation in Aug.). The mean values correspond to the periods of fig. 7.

	Frost winter situation [mean]	Hot summer situation [mean]
air temperature [°C]	-4.1 (±3)	19.8 (±5)
diel PAR [µmol photons m^{-2} s^{-1}]	44 (±145)	497 (±657)
PAR daily photon dose [mol m^{-2}]	3.9 (±12)	43.0 (±57)
effective heat demand in winter and heat removal in summer [Watt m^{-2} of PBR surplus]	283 (±56)	147 (±205)
mean heat demand/removal [kWh d^{-1}]	6.8 (±1.3)	3.5 (±4.9)

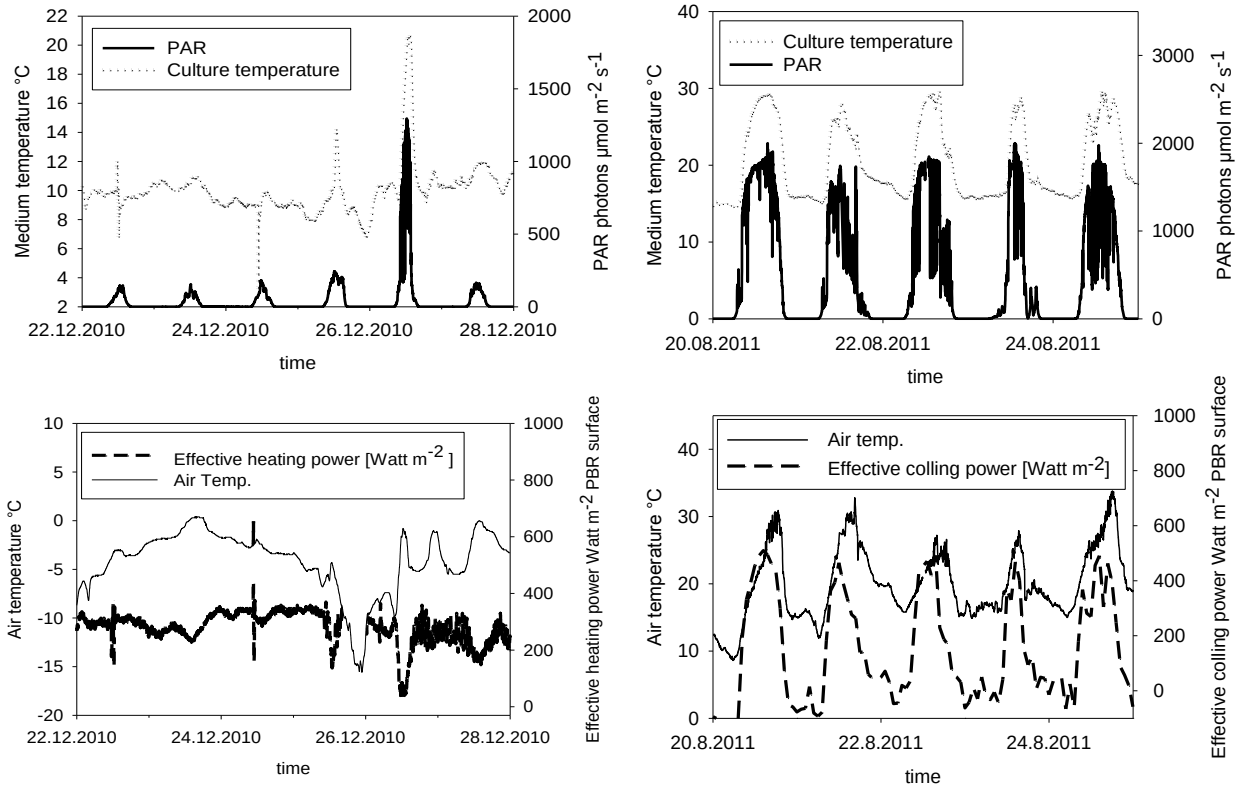

Fig. 7 Typical condition of heat demand during winter (left) and heat surplus which has to be removed during summer cultivation (right). The energy was calculated based on the temperature difference between outflow and inlet of the culture medium into the PBR units, assuming a heat capacity of the culture medium of 4.18 J g^{-1} K^{-1} and a density of 1 g ml^{-1}.

Of course the heat conduction properties of the PBR material influences mainly the required waste heat demand or for the summer situation the amount of surplus heat which has to be removed in order to prevent a overheating of the microalgae. To improve the energy demand of waste heat prototypes of isolated (double

glass) PBRs were tested offline (no circulation of the culture medium). For non-isolated (plastic material) PBRs a heat power of 112 Watt m^{-2} was necessary to increase the temperature by 16°C in comparison to the ambient air temperature. This waste heat demand was reduced to 66 Watt m^{-2} of PBR surface, if the microalgae were cultured in double glass isolated PBRs. Thus, isolation of PBR decreased the waste heat demand nearly twofold or in other words facilitates a doubled temperature controlled PBR surface with the same amount of waste energy. Wind and rain effects were not taken into account but should be considered for dimensioning of heat exchanger capacity. These data are mandatory for the up scaled cultivation plants and year round production of microalgae in PBRs at latitudes which strong fluctuation of the ambient temperature and winter temperature below 0°C.

Chapter 3 Influence of mixing and shear stress on Chlorella vulgaris, Scenedesmus obliquus, and Chlamydomonas reinhardtii

The complete publication is provided in the attachment.

This chapter has been published as: Leupold M., Hindersin S., Gust G., Kerner M., Hanelt D. (2012) Influence of mixing and shear stress on *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlamydomonas reinhardtii*. Journal of Applied Phycology: DOI 10.1007/s10811-012-9882-5.

Chapter 4 Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors

The complete publication is provided in the attachment.

This chapter has been published as: Hindersin S., Leupold M., Kerner M., Hanelt D. 2012. Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors. Bioprocess Biosyst Eng: DOI 10.1007/s00449-012-0790-5.

Chapter 5 Key parameters for outdoor biomass production of Scenedesmus obliquus in solar tracked photobioreactors during different seasons

The complete publication is provided in the attachment.

This chapter has been submitted to the journal of Biomass and Bioenergy as: Hindersin S., Leupold M., Kerner M., Hanelt D. 2012. Key parameters for outdoor biomass production of *Scenedesmus obliquus* in solar tracked photobioreactors during different seasons. Nr. JBB-S-12-01227-2

Chapter 6 Influence of nitrogen limitation on the photosynthetic efficiency of Scenedesmus obliquus

Introduction

The availability of nutrients to the microalgae determines both the productivity and the cellular biomass composition. To prevent limitation of productivity the N-concentration of the culture medium should exceed 25 mg L^{-1} for *Scenedesmus sp.* (Mostert and Grobbelaar 1987). For the modification of the cellular composition N-limitation can be used to induce carbon storage product accumulation of starch and lipids (Dragone et al. 2011; Harrison and Griffiths 2009). However, the limitation stress results in the decrease of growth rates and affects photosynthetic efficiencies (White et al. 2011). Thus, the starch or lipid contents are negatively correlated to the growth rates of the microalgae (Williams and Laurens 2010). If the primary products of photosynthesis are not used to generate new daughter cells (constant cell concentration) there might be a constant biomass yield on PAR (increasing cell size) during the shift from N-sufficient growth to initial N-limitation. To test the hypothesis the following N-limitations experiments were conducted outdoors including the determination of the photosynthetic performance and the biomass yield on PAR.

Materials and methods

The experiments were performed at the PBR pilot plant which had been described in Chapter 4 and 5. Two production units (each with a photosynthetic active surface of 4 m^2) were inoculated with *S. obliquus* to CDW of 0.5 g L^{-1} and cultured separately. At the beginning of the cultivation both cultures had nitrogen fertilizer concentration of 100 mg NO_3^--N L^{-1}. For nitrogen limitation one culture were operated as batch culture without further addition of N-source and the second as N-sufficient control in fed batch mode. In the N-supplied fed batch culture the nitrate consumption was automatically compensated by addition of fresh medium to a set value concentration of 100 mg NO_3^--N L^{-1}. The biomass yield on PAR g CDW mol photons^{-1} was calculated from the formation of biomass during 24 hours following the method described in Chapter 5. The photosynthetic activity of the microalgal cultures was measured using pulse amplitude modulation (PAM) fluorometry and the method of rapid light curves as described in Chapter 2. For determination of carbon and nitrogen content of the biomass, a CN-Analyzer (TOC V_{CPN}, Shimazu, Japan) was used. The culture medium was filtered by 0.45 µm pore size and the dissolved organic carbon (DOC) and dissolved nitrogen (DN) were analyzed. To measure C and N contents the sample where diluted to below concentration of 500 mg C L^{-1} and 333 mg N L^{-1} following the protocols (NPOC and TN of the TOC V_{CPN}, Shimazu,

Japan). In addition C and N contents of the complete cultures was determined. The difference between the total organic carbon (TOC) and the DOC and the difference between the total nitrogen (TN) and the DN were used to calculate the amount of carbon and nitrogen per cell. Reinstall of the nitrate feeding for the N-limited cells were applied to study the kinetics of the recovery of photosynthetic and growth parameters after N-limitation.

Results and discussion

S. obliquus was grown outdoors under nitrogen limited and nitrogen saturated conditions in photobioreactors. The light fluctuated from PAR below 10 mol photons m^{-2} d^{-1} for cloudy days up to 50 mol photons m^{-2} d^{-1} for clear weather conditions. After three days of cultivation all available nitrate was consumed and the microalgae were N-limited for a period of six days followed by reinstall of the N-feeding (Fig. 8 -10 A) in contrast to the N-supplied culture (Fig. 8-10 B).

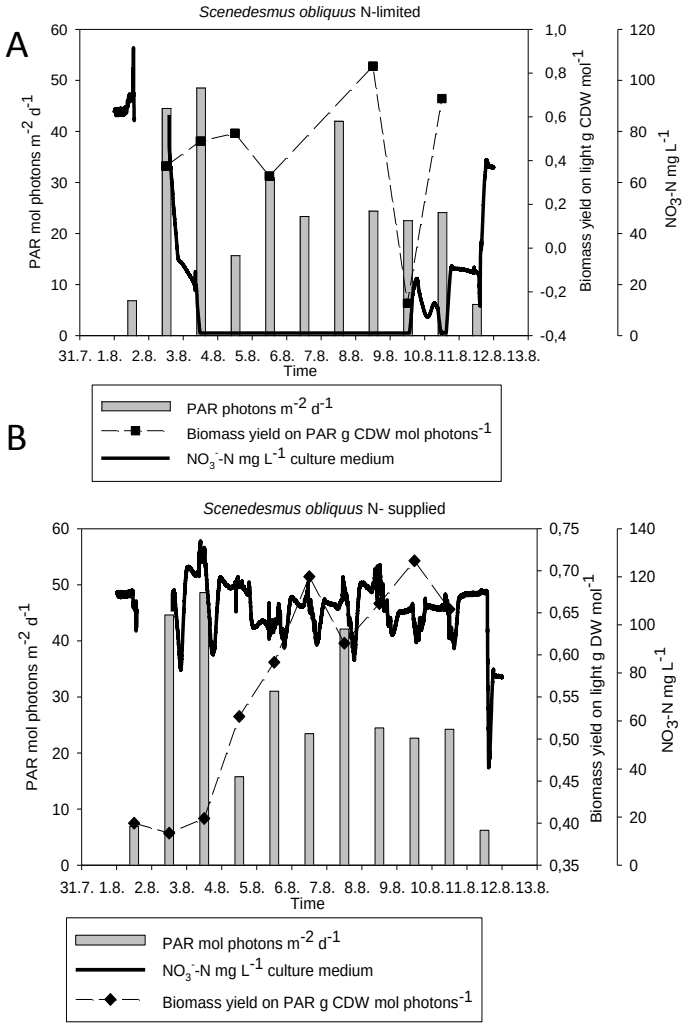

Fig. 8 The biomass yield of *Scenedesmus obliquus* under nitrogen limitation (A) and nitrogen supplied conditions (B).

Interestingly at the first days of the experiment the biomass yield of the N-limited *S. obliquus* cells were higher than of the N-supplied *S. obliquus* cells (Fig. 8 A, B). Also at N-limitation phase the biomass yield remained equivalent or higher than under N-supplied conditions. This might be related to a equal biomass yield on PAR if photosynthesis mostly contributes to storage products like starch (Brányiková et al. 2011). Energy consuming processes like nitrated reduction for protein synthesis and the generation of new cells could have been reduced (Brányiková et al. 2011). However, at the day of the reinstall of the N-feeding the biomass yield decreased below values of -0.2 g CDW mol photons^{-1}, indicating that more storage products were consumed than produced by the metabolism for generation of new N-containing cell compounds like pigments and proteins (Brányiková et al. 2011).

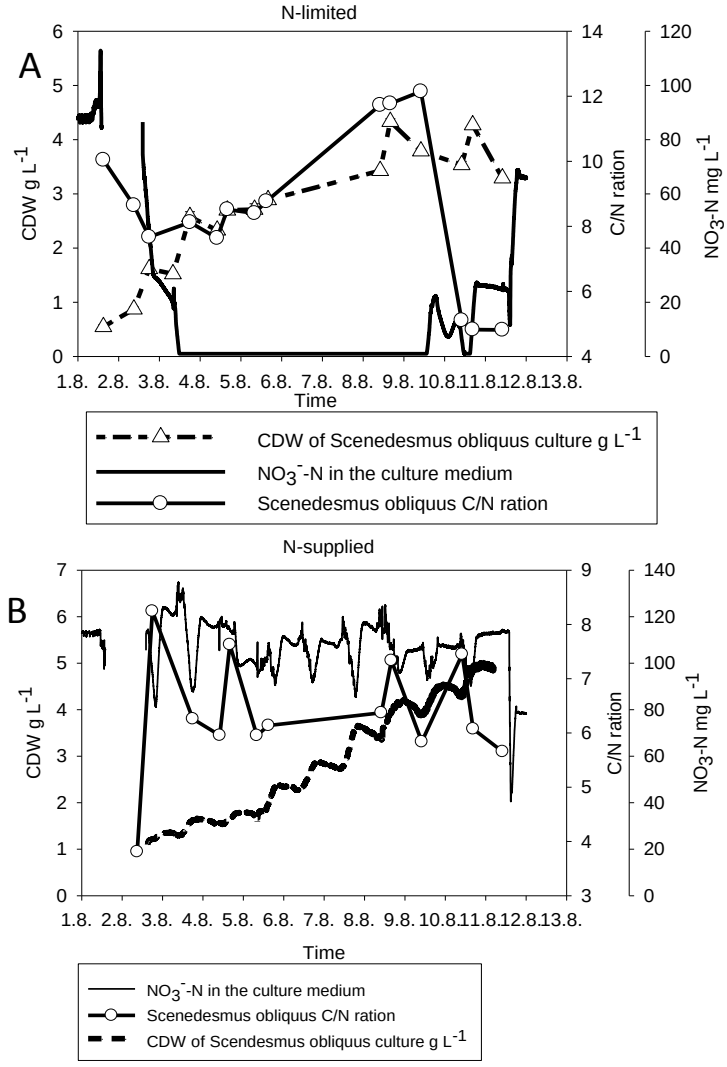

Fig. 9 Carbon/Nitrogen ration (C/N ration) of the *Scenedesmus obliquus* dry biomass under nitrogen limitation (A) and nitrogen supplied conditions (B).

At N-supplied conditions the C/N ration of the *S. obliquus* cell varied between 4 to 8. In contrast the C/N ration increased to 12 by the accumulation of carbon storage products and decrease within one day to 5 after the reinstall of the N-Feeding (fig. 9 A, B).

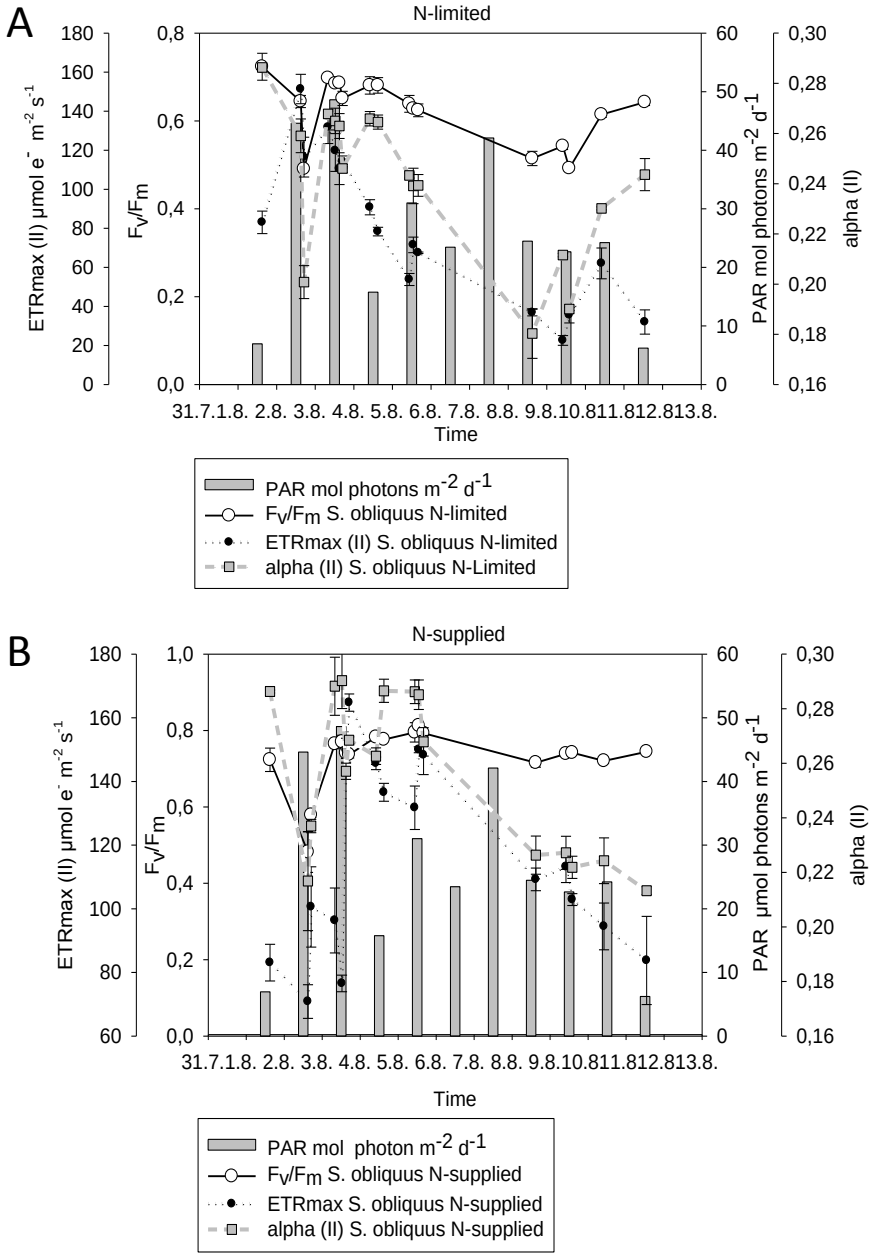

Fig. 10 Photosynthetic parameter of the *Scenedesmus obliquus* under nitrogen limitation (A) and nitrogen supplied conditions (B).

At the first days of cultivation the maximum quantum yield (F_v/F_m) decreased from 0.75 to values of 0.5 for both N-limited and N-supplied conditions due to low self shading by low CDW concentrations and high PAR intensities (Fig. 10 A, B). During the N-limitation phase the F_v/F_m again decreased to 0.5 while in the N-supplied culture no reduction of the F_v/F_m was detected. Alpha (II) decreased to lowest values of 0.18 at N-limited conditions and recovered to levels of the N-supplied culture after reinstall of the N-feeding. Alpha (II) of the N-supplied culture also decreased during the cultivation above 2.5 g CDW L^{-1}. The overall trend represented a low light acclimation which was expected because the culture was operated in batch mode. The ETR_{max} of both cultures started with values of about 80 µmol $e^- m^{-2} s^{-1}$ and increased to levels of 160 µmol $e^- m^{-2} s^{-1}$. Low light acclimation lowered the ETR_{max} for both culture conditions so that this parameter indicated low light acclimation.

The results revealed that C-accumulation is possible without a reduction of the biomass yield if the N-limitation does not exceed a doubled C/N ratio of the biomass in comparison to unlimited growth. With respect to the detected decrease in the photosynthetic parameter this seems contradictory. However, the respiration rate might be decreased once photosynthesis contributes only to C-accumulation and energy consuming processes in the metabolism might have been kept to minimal levels (Brányiková et al. 2011).

Chapter 7 Discussion

Optimization of airlift mixing

The price composition of the produced biomass revealed which component obtains the greatest potential for optimization to reach economic production of microalgal biomass for bulk products like feed and biofuels. An estimation of the economics suggests that the effort for mixing contributes 3.1 € kg^{-1} to the overall price of 5.9 € kg^{-1} produced in flat panel PBRs (Norsker et al. 2011). Generally, mixing is needed for mass transfer, prevention of cell conglomeration and light/dark oscillation of the cell within the light path of the PBR (chapter 3, fig. 2)(Borowitzka 1999). In the present study the air flow per liter of culture medium (vvm) for lift mixing was 0.4 $LL^{-1}min^{-1}$ (chapter 4, 5) by injection of air pulses at a constant frequency of 0.25 hz. Furthermore, high oversaturation of oxygen at maximum productivity was prevented by flue gas supply (about 10% CO_2 of flue gas)(Chapter 4). The RLCs of the microalgae can be used to reduce the mixing because they give an insight into the current photosynthetic state of the culture (fig. 1). For *S. obliquus* maximum I_k (II) values of 510 µmol photons m^{-2} s^{-1} were found (fig. 4). The mean annual PAR was 250 µmol photons m^{-2} s^{-1} and thus the mean PAR during a 12 hour day roughly corresponds to 500 µmol photons m^{-2} s^{-1}. Consequently, this light intensity is in a range of high photosynthetic efficiency (high alpha at optimum CDW) and "light dilution" by mixing is not needed during periods of low or medium PAR intensities which are below the I_k value of the microalgae. Therefore following assumptions are made to optimize airlift mixing:

- Light dilution by fast light/dark oscillation of the cells is dispensable until the irradiance at which alpha of light exposed cells starts to flatten from linear increase (fig. 1)
- Mass transfer rates for O_2 removal, and CO_2 and nutrient uptake depends on productivity
- Fastest mixing ($f_{airlift\ max}$) is only necessarily during maximum PAR
- Minimum mixing ($f_{airlift\ min}$) is obligatory to prevent anaerobic culture conditions at night, settling and conglomeration of cells

In conclusion, mixing frequency ($f_{airlift}$) can be drastically optimized by modulating it with respect to ambient PAR with the following equations.

$$f_{airlift\ (logarithmic)} = \mathbf{LN(PAR)} + f_{airlift\ min} \quad (1)$$

$$f_{airlift\ (linear)} = \mathbf{0{,}00011(PAR)} + f_{airlift\ min} \quad (2)$$

$$f_{airlift\ (exponential)} = f_{airlift\ max} * \mathbf{EXP((PAR\ -2000)/500))} + f_{airlift\ min} \quad (3)$$

The logarithmic approach would result mainly in decreased mixing during the night, while mixing during the day would be close to the maximum (equation 1). An airlift modulation by exponential dependency of PAR as suggested by equation 3 has the highest potential for conserving energy (Fig. 9). Also the linear approach may already reduce the power consumption by the factor four: Based on the intersection point of equation 2 and mean PAR of 250 µmol photons m^{-2} s^{-1} the gas flow rate vvm would be lowered from 0.4 to 0.1 $LL^{-1}min^{-1}$.

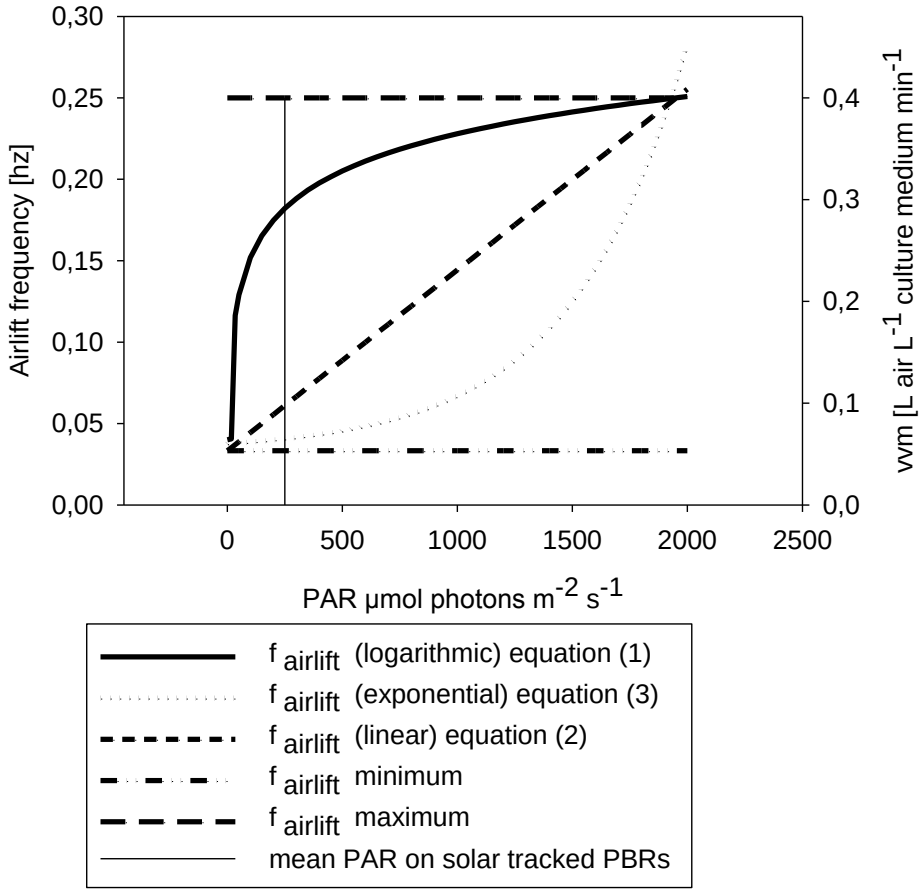

Fig. 9 Modulation of the airlift frequency is given as dependency of PAR to optimize energy consumption of the cultivation process using the annual mean PAR measured at the pilot plant (chapters 4, 5). For comparison the vvm is shown on right y-axis. ($f_{airlift\ max}$ = 0.4 $LL^{-1}min^{-1}$ (vvm)).

Optimized cell harvesting

An operation of PBRs at the optimum biomass concentration and not at the maximum possible for a specific system relies on an energy efficient harvesting concept because more culture volume is needed to be processed (chapter 5). Sample dehydration , water and nutrient recycling all play a major role in the downstream processing and water footprints (Yang et al. 2011). Hence, dissolved air flotation and suspended air flotation are possible solutions and in combination with flocculation (by

high pH values) they will improve the system's energy balance (Grima et al. 2003; Sukenik and Shelef 1984; Uduman et al. 2010; Vandamme et al. 2010; Vandamme et al. 2011; Wiley et al. 2009). Flocculation can be enhanced by increasing the pH of the medium (Wu et al. 2012). By interrupting the flue gas supply during light exposure photosynthetic alkalization increased the pH from 7 to over 10 within one hour. A combination of photosynthetic alkalization and dissolved air flotation represent a potential way to harvest microalgae more energy efficient (Fig. 10). However; further studies are essential to elucidate the potential for large scale operation.

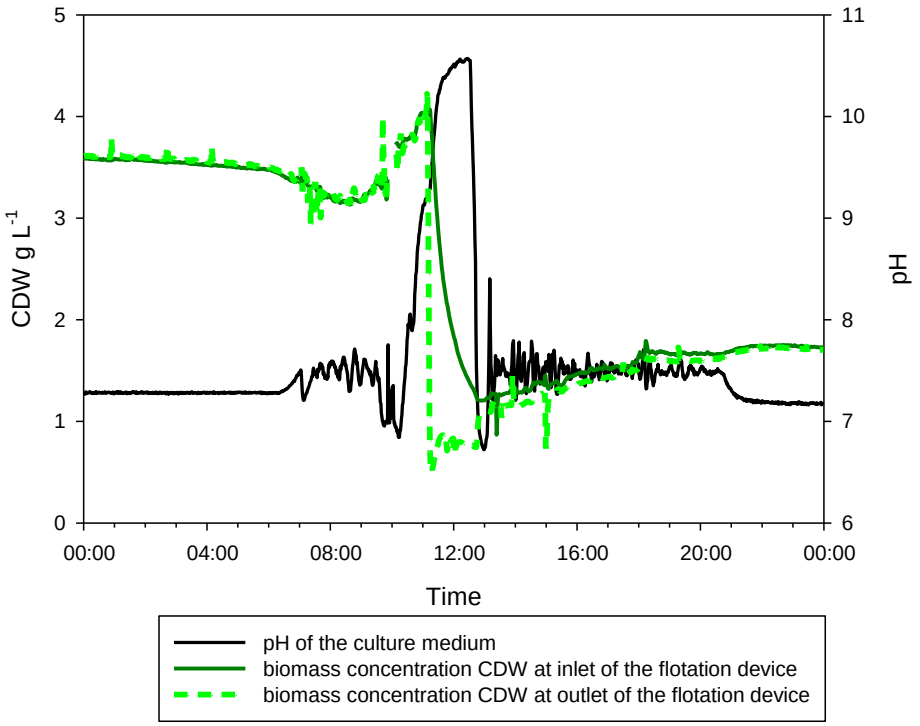

Fig 10. Biomass concentration of *Scenedesmus obliquus* culture is given including a cell harvesting during midday by dissolved air flotation and photosynthetic alkalization during midday. Flue gas supply was interrupted followed by an increase of pH and microalgal biomass was partially harvested from 11:00h to 13:00h.

In this study typical daily cycle of cell growth were observed for *S. obliquus* (chapter 5). Similarly the cell diameter of *C. vulgaris* increased during the day followed by a decrease at night due to the cell dividing activity which was indicated by the increase in the cell numbers (fig. 13). Furthermore, in literature accumulation of C- storage products was found, which increases the C/N ration during the day (Ma et al. 1997). Hence, both the increased cell diameter and the increased C/N ration leads to

the conclusion that the optimum harvesting time would be at the end of the light period. Additionally the respiratory losses would be reduced because of the lower biomass concentration.

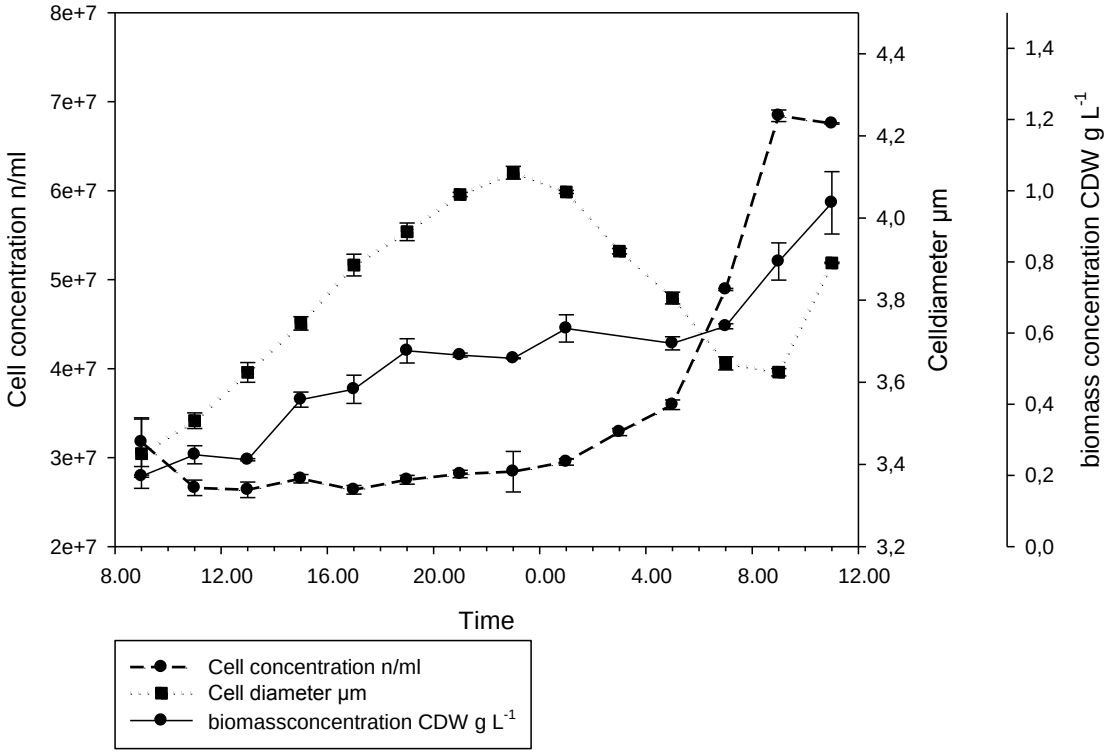

Fig. 11 Typical diurnal cycle of the cell dividing activity during night, cell growth and biomass increase during day of *C. vulgaris* in outdoor cultivation.

This cumulative dissertation evaluates photosynthetic efficiency of microalgal mass cultures and specific aspect for the optimization of microalgal biomass production. The novel application of solar tracked photobioreactors and the potential of year round biomass production by the use of waste heat are presented, resulting in a combination of engineering and biological science.

To supply the elementary basis for cell compounds there is no doubt that carbon is the most important one to contribute roughly to 50 % of the CDW (Lardon et al. 2009; Shen et al. 2010). Therefore, the successful integration of flue gas as the only carbon source might improve the overall economics of microalgal production (Chapter 5). High oversaturation of oxygen is often found to inhibit the productivity in closed PBRs (Grobbelaar 1994; Grobbelaar 2009). Here flue gas containing 10 % CO_2 combined with a high gas flow rate of 0.4 $LL^{-1}min^{-1}$ limited the oxygen levels to below 20 mg L^{-1} (chapters 4, 5).

It has been demonstrated how temperature affects microalgal photosynthesis and clear temperature dependencies can be found for specific strains (chapters 2, 5). For outdoor cultures without an artificial light source the situation is more complex because the productivity over 24 hours is the sum of the daily production and the total biomass losses in the night. The waste heat supply enabled the continuous cultivation in northern latitudes where strong variations in daily mean air temperatures between -5°C and 27°C occurred during winter and summer. These variations were reduced in the outdoor cultivation by heating and cooling and temperature in the culture medium was always maintained at a daily mean of 9°C - 25°C (chapter 5). Increased day temperatures resulted in increased productivities but are connected to high demands of waste heat. Isolated glass PBRs halved the waste heat supply which facilitates the temperature control to the species specific optimum. Strain selection will also improve the productivity, i.e. if cryophilic microalgae are cultivated in outdoor PBRs during winter because of their superior photosynthetic activity at low temperatures in comparison to mesophilic strains (chapter 2). Furthermore the species-specific optimum temperatures may be used to install a culture conditions in which the strain of interest outcompetes alien species by higher growth rates (chapter 2).

In chapter 5 the overall mean temperature of the *S. obliquus* cultivation was 19°C which is 11°C below the optimum temperature of 30°C (chapter 2). In accordance with the literature, at these suboptimal culture temperatures a mean value of $Y_{x,E}$ of 0.41 g CDW mol $photons^{-1}$ was determined. This temperature induced decrease of the biomass yield might be compensated by improvements of the temperature course during the day. This should include low night temperature of the culture which revealed to increase the productivity (chapter 5). It is most likely that this based on the

decreased respiration rate which is temperature dependent and can be described by the Arrhenius relationships (Grobbelaar and Soeder 1985). The respiration rate in combination with PAR also determined the optimum biomass concentration (chapter 5). At low PAR of ≤ 10 mol photons m^{-2} d^{-1} and high CDW above 5 g L^{-1} the respiration rate was higher than the biomass production during the day. Hence, in these situations either the PAR should be increase or the CDW decreased, which is the only possibility for outdoor culture without artificial light supply. High outdoor irradiance up to full sunlight 2000 µmol photons m^{-2} s^{-1} can be applied without photoinhibition by adequate self shading and mixing (chapter 4). However, analysis of the biomass yield over 24 hours indicated light saturation at high constant PAR (chapter 5, equation (2) and (3)). For adequate light/dark cycles mixing is required but shear stress induced by mixing affects the photosynthetic activity (chapter 3). For robust microalgal the positive effect of enhanced mass transfer and lower boundary layer by mixing supports higher growth rates (Grobbelaar 1994). For *S. obliquus* and *C. vulgaris* optimum tip speed of 126 cm s^{-1} were found which should be considered by optimization of the PBR design. In contrast, mixing did not increase the photosynthetic activity of the morphological sensitive *C. reinhardtii*.

With solar tracked PBRs, the microalgal cultures are exposed to higher irradiance (up to 45%) than in a static system, which leads to a higher optimum biomass concentration and volumetric productivity (chapter 4 and 5). The controlled light supply in the “offset mode” enables low irradiance at low cell densities at the initial outdoor cultivation phase after inoculation (e.g. a small volume of preculture). This eliminates the necessity for additional shading or step wise inoculation, as compared with other outdoor systems. 78 % of the annual biomass production is generated between March and September due to the solar energy distribution within the year (chapter 5). Therefore the question remains if the economic effort for temperature regulation in winter is compensated by any commercial products derived from microalgal biomass. In conclusion the most important key parameters for process optimization are continuous adjustment of the biomass concentration and the culture temperature with respect to the fluctuating weather conditions. This optimization of the key parameters resulted in a threefold increase of biomass productivity. Finally microalgae might become an alternative to traditional biomass sources based on agricultural crop land. To reach this goal process optimization is essential as suggested in chapter 7 and the presented study contributes by adding new information about temperature regulation, aspects of photosynthetic efficiencies and microalgal culture operation of photobioreactors.

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Abbreviations

ETR (I)	electron transport rate of the photosystem I
ETR (II)	electron transport rate of the photosystem II
ETR_{max}(I)	maximum electron transport rate of the photosystem I
ETR_{max}(II)	maximum electron transport rate of the photosystem II
I_k(I)	saturating irradiance for the photosystem I
I_k(II)	saturating irradiance for the photosystem II
K_a	light absorption coefficient [m^{-1}] of the specific microalgal culture
L	light path within the photobioreacto [cm]
PS (I)	photosystem I
PS (II)	photosystem II
CDW	cell dry weight, [g]
OBC	optimum biomass concentration [g L^{-1}]
OLP	optical light path [mm]
PAR	photosynthetic active radiation (µmol photons m^{-2} s^{-1}, 400–700 nm); horizontal PAR (PAR absorbed by a horizontal plane)
PAR_0	incident photosynthetic active radiation at the photobioreactor surface

PAR_{24h}	diel PAR mol photons m^{-2} d^{-1}
PBR	photobioreactor
PE	Photosynthetic efficiency % (Energy content of the generated biomass/Energy of supplied light)
R_{12h}	Respiration (including all biomass losses) in 12 hours of darkness g L^{-1}
RLC	rapid light curve
V	culture volume [L]
vvm	gas flow rate (L of air/flue gas per L of culture medium and minute)
X_{CDW}	biomass concentration, [g L^{-1}]
$Y_{x,E}$	biomass yield (biomass formation on PAR photons) g CDW mol $photons^{-1}$
Y_{24h}	biomass yield over 24h (including biomass loss during the night)

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Chapter 3 Influence of mixing and shear stress on Chlorella vulgaris, Scenedesmus obliquus, and Chlamydomonas reinhardtii

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The author of this dissertation contributed by the isolation of the *Scenedesmus obliquus*, experiments for optimum culture conditions and the development of the monitoring of photothynthetic activity via *insitu*-PAM method in highly mixed photobioreactors. Furthermore the data were analyzed and the manuscript was finished for publication.

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Influence of mixing and shear stress on *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Chlamydomonas reinhardtii*

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Abstract Photosynthetic activity (PA) and growth of different microalgae species (*Chlorella vulgaris, Scenedesmus obliquus*, and *Chlamydomonas reinhardtii*) depends in addition to other factors on mixing (tip speed) and shear stress (friction velocity) and was studied in a stirring tank (microcosm). In order to detect cause–effect relationships for an increase in photosynthetic activity, experiments were conducted under different pH values (6.0–8.5) and CO_2 concentrations (0.038 and 4 % (*v*/*v*)). The PA was determined as the effective quantum yield by pulse amplitude modulation during a stepwise increase of the tip speed from 0 to 589 cm s^{-1} (friction velocity: 0–6.05 cm s^{-1}) in short-term experiments. The increase caused a distinctive pattern of PA of each species. Compared to 0 cm s^{-1}, *C. vulgaris* and *S. obliquus* showed a 4.0 and 4.8 % higher PA at the optimum tip speed of 126 cm s^{-1} (friction velocity of 2.09 cm s^{-1}) and a 48 and 71 % higher growth, respectively. At 203 cm s^{-1}, the PA dropped to the value of the unstirred control, while at 589 cm s^{-1}, the PA decreased of up to 7 and 8 %. In contrast, *C. reinhardtii* showed 7 % stronger growth at 126 cm s^{-1}, while the PA decreased about 15 % at an increase of tip speed to 589 cm s^{-1}. For all investigated microalgae, the pattern of PA and higher growth was not only explained by the main contributing factors like light supply, nutrient supply, and overcoming diffusion gradients. The results indicate that hydrodynamic forces have a stimulating effect on the physiological processes within the cells.

Keywords Mixing · Shear stress/friction velocity · Microalgae · Chlorophyta · Photosynthetic activity · Growth · Photobioreactors

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Introduction

For the cultivation of microalgae with high growth rates on an industrial scale, closed photobioreactors (PBRs) or open ponds are needed (Borowitzka 1999). In such systems, mixing of the culture medium by pumps, airlift systems, or paddles is indispensable. The mixing generates shear forces which have different influences on microalgae cultures. Shear forces are generated by the pumps, the PBR construction (i.e., sharp edges, corners, or walls) and flow of the culture medium, or by bursting of airlift bubbles. For every possible microalgal cultivation device (i.e., gas-agitated bioreactors, mechanically stirred vessels, pipework and flow channels, or turbulent jets) the shear forces are described (Chisti 2001). On one hand, smooth shear forces due to mixing are indispensible and enhance microalgae growth. On the other hand, very high shear forces as well as rapid pressure changes can also damage microalgal cells (Bronnenmeier and Märkl 1982).

It is known that algal species react differently to mixing; each has its own mixing optimum and thus needs its specific mixing and cultivation device (Richmond 2007). The degree of mixing depends also on the concentration of the cells (Märkl 1980). Without mixing, a high cell density culture cannot be maintained and would lead to cell clumping into aggregates. This, in turn, develops into a three-phase system (gas/liquid/solid) inside the PBRs, which is prone to decreasing mass transfer rates (Panda et al. 1989). Furthermore, with

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increasing cell density, it becomes more difficult to maintain homogeneous suspension of cell cultures so that mixing needs to be increased (Carvalho et al. 2006). Mixing leads also to a homogeneous distribution of the dissolved substances (e.g., nutrients) which favors mass transfer in and out of the cells (Contreras et al. 1998) and maintains a homogenous pH value (Kommareddy and Anderson 2005). Mixing is a method to overcome limitation and diffusion gradients (Richmond 2007), as well as boundary layers. The uptake of nutrients by algae, especially nitrate, was enhanced in an open-pond system with a mixing through paddles turning at angular frequency greater than ω=5 rad s^{-1} (Pasiack and Gavis 1975). Furthermore, a higher photosynthetic yield, growth rate, and productivity (+50 %) was found in an open pond when doubling the mixing rate by increasing flow velocity to 30 cm s^{-1} (Richmond and Vonshak 1978). Gas transfer, i.e., CO_2 supply and the outgassing of O_2, can be enhanced with mixing as well. Finally, the heat transfer in the medium is optimized, as e.g., a thermal amplitude of up to 8 °C has been recorded between the top and bottom of unmixed cultures (Oswald 1988). Moreover, light supply is influenced by mixing, because of the more even irradiation pattern of each single cell, depending on the cell concentration and light path (Sobczuk et al. 2006). Unfortunately, all studies cited above investigated the effects of mixing, with an undefined value of shear force. Hence, in PBRs, the flow field observed generates normal and tangential components of the stress tensor (Schlichting 1968), which manifest themselves as pressure and as shear forces (or shear stress) in the fluid and at the geometric (wetted) boundaries of the device such as walls or bottom. The generated turbulent flow in PBRs can be classified by the four statistical moments from measured flow time series. Turbulence intensity can thus be connected to Reynolds stress, wall shear stress and mean flow speed for steady operational conditions, or to numerical models when used to identify the prevailing flow conditions (Tennekes and Lumley 1972). This allows finally selecting the optimal magnitudes of the hydrodynamic parameters acting on microalgae. Shear forces are related to shear rate (s^{-1}) and shear stress (Pa) which is common in biotechnology (Chisti 2001; Sánchez Pérez et al. 2006). Shear stress can be subdivided into Reynolds stress (in turbulent flows) and wall shear stress (between fluid and wall), often expressed as friction velocity (cm s^{-1}) in oceanography, sedimentology, and geochemistry (Thomsen and Gust 2000; Kleeberg et al. 2007). Microalgae are smaller than the Kolmogorov inertial-viscous length scale, and shear stress can affect them between fluid and wall and not because of the turbulence in the fluid (Thomas and Gibson 1990). In this study, tip speed and friction velocity were used as parameters to represent mixing and the shear forces applied. The positive effect of using a known shear force, in this case shear stress, on microalgae is described by Hosaka et al. (1995), where oxygen production of *C. vulgaris* decreased due to short-time shear stress of 0–0.5 Pa, whereas in long-term experiments, growth was enhanced. In contrast, the blue-green alga *Spirulina* showed a reverse relationship of oxygen production and growth under same hydrodynamic stimuli (Hosaka et al. 1995).

Thomas and Gibson (1990) created a scale with organism from different taxonomic groups, showing relative sensitivities towards shear forces from high to low: *green algae* < *cyanobacteria* < *diatoms* < *dinoflagellates*. As can be observed from this scale, coccal green algae are most resistant to shear force, which is due to their special morphology, cell wall, and size. This is one of the reasons why green algae are among the most frequently used organisms in PBRs. Violent mixing, which causes high shear stress, affects the algal morphology, especially the cell walls. This was discovered with cell wall mutants of *Chlamydomonas* (Gudin and Chaumont 1991). It was also shown that cyanobacteria and colonies of diatoms developed shorter filaments or chain lengths when cultures were strongly mixed (Thomas and Gibson 1990). Shear forces due to mixing with different types of pumps can also damage the flagella of microalgae, for example of *Tetraselmis suecica* (Jaouen et al. 1999). In fact, high shear forces even kill microalgae. By the application of locust bean gum, high forces of about 10 Pa were reached and turned out to kill the cells of the diatom *Chaetoceros muelleri* in a special shear tank. Moreover, it was observed that shear force responses on cells were dependent on the exposure time (Michels et al. 2010), and algae can even adapt to this kind of stress.

The studies cited above clearly show that required type and strength of shear forces have to be considered in the design and optimization of PBRs. Mixing within a PBR, when analyzed for the hydrodynamic parameters active on microalgae suspended, indicates that different designs produce different stimuli for the algae. Threshold values for optimum shear forces have to be determined because they are difficult to derive from the literature. Common studies about mixing or stirring are specific on the used device or a sum parameter, where shear forces were applied, but only very few values of shear forces applied are reported and no reproducible data sets for different cultivation devices have been established or pointed out. We selected tip speed (TS) as a parameter for mixing and bottom wall shear stress, expressed as friction velocity (u*) by which we show that a calibrated stirring tank (the microcosm; (Gust 1989)) can be used to check the influence in case of growth and photosynthetic activity on microalgae. With this hydrodynamically calibrated system, it is possible to generate reproducible data. Shear stress and friction velocity is also generated at all surfaces with a relative velocity of the wetting liquid and also at rising bubbles (Sánchez Pérez et al. 2006). This is considered as a

calibration parameter for the active shear force here and in any cultivation system of a high surface-to-volume ratio, especially in PBRs. In order to determine threshold values for optimum cultivation settings, the effects of different friction velocities were measured at high resolution in time by the effective quantum yield Φ_{PSII} of photosynthesis and for long-term experiments by growth.

Material and methods

Algal strains

The experiments were done with four different algae strains. *Scenedesmus obliquus* (No. U169), *Chlorella vulgaris minima* (No. U126) and *Chlamydomonas reinhardtii* (No. U170) were obtained from the SVCK algal collection at the University of Hamburg. *Aphanizomenon flos-aquae* was obtained from the SAG Culture Collection at the University of Göttingen (SAG 31.87).

Medium and cultivation

The medium for *C. vulgaris* or *S. obliquus* was composed of 2 g L^{-1} Flory Basis Fertilizer 1 (Euflor, Germany) and 3.22 g L^{-1} KNO_3; pH: 7.0. The medium for *C. reinhardtii* was composed of 2 g L^{-1} Flory Basis Fertilizer 1 (Euflor, Germany) and 2 g L^{-1} NH_4Cl (Growth enhancer 1 mL L^{-1} Acetate); pH: 7.0. For *A. flos-aquae*, modified BG11 medium was used which consisted 6 mg L^{-1} citric acid, 6 mg L^{-1} ferric ammonium citrate, 1 mg L^{-1} EDTA, 1,500 mg L^{-1} $NaNO_3$, 41 mg L^{-1} $K_2HPO_4 \cdot 3H_2O$, 75 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 38 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 40 mg L^{-1} Na_2CO_3, 2.86 mg L^{-1} H_3BO_3, 1.81 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 0.222 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.391 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 0.079 mg L^{-1} $CuSO_4 \cdot 5H_2O$, and 0.049 mg L^{-1} $Co(NO_3)_2 \cdot 6H_2O$; pH: 7.5.

Microalgae were precultured from a 50-mL flask of the SVCK algal collection in 5-L Schott glass flasks, filled with 4 L of algae-specific medium. After 10 days of cultivation at 20 °C, with an irradiation of 100 μmol photons m^{-2} s^{-1} and aeration with CO_2-enriched air (4 % *v*/*v*), the experiments within the microcosm were started. The preculture was not stirred to avoid any adaptation to shear forces. A slight mixing was achieved by bubbling with the CO_2-enriched air. *A. flos-aquae* was precultured in a 2-L flask and cultivated with identical parameters as the other microalgae, except for the temperature, which was kept at 25 °C. The pH was adjusted in general to pH 7.0 and to 7.5 for *A. flos-aquae*. Microscopic analyses were done using a standard Zeiss microscope at ×400 magnification.

Analytics

Cell density, cell dry weight, and growth rate

Cell growth was monitored by measuring the optical density at 750 nm (OD_{750}). Cell dry weight (CDW) was determined gravimetrically in pre-dried 50-mL tubes. The 50-mL culture was centrifuged for 30 min at 6000×*g*; the supernatant was discarded and the pellet was dried at 85 °C for 24 h. The CDW (g L^{-1}) was calculated from the difference in weight of the pre-dried tube and the tube with dried pellet. The specific growth rate μ_{spec} (day^{-1}) was calculated as the natural logarithm of two CDW values in g L^{-1} divided by the time (d) (Eq. 1).

$$\mu_{spec} = \frac{\ln(CDW_{end}) - ln(CDW_{start})}{t_{end} - t_{start}} \quad (1)$$

Measurement of the effective quantum yield (Φ_{PSII})

Effective quantum yield (Genty et al. 1989) of the culture within the microcosm was determined every 10 seconds by online fluorescence measurements at the surface of the microcosm using the saturation pulse method and measuring light at high frequency of 400 KHz for detection of the minimum and maximum fluorescence (F_0', F_m'). To allow in situ measurements, the detector of a Dual-PAM 100 (Walz, Germany) was placed on the surface of the outer cylinder at the bottom of the microcosm, where mixing and calibrated friction velocity occurs. For determination of F_m' a maximum saturation pulse of ~2,000 μmol photons m^{-2} s^{-1} and a pulse length of 300 ms was applied. The measurements were done during irradiation; thus, the effective quantum yield of the light-adapted state of photosynthesis in the algae was determined. Due to low irradiance, Φ_{PSII} remained close to the value of the maximum quantum yield F_v/F_m measured after dark phase. The effective quantum yield enables the estimation of the efficiency of photosynthesis in situ. If only a part of the absorbed light can be used for photochemistry of photosynthesis, the rest of light will be discharged by non-photochemical quenching of the microalgae. This method enables to detect positive or negative effects per selected mixing and friction velocity on photosynthetic activity of the microalgae. For the cyanobacterium *A. flos-aquae*, unfortunately, the measurements of Φ_{PSII} was not possible with the Dual-PAM technique, as pigment content and morphology of its photosynthetic apparatus differs largely from that of green algae.

Microcosm

The microcosm used (Gust 1989; Huettel and Gust 1992; Gust and Müller 1997) was a 2.8-L closed system, operated

with 1.75-L microalgae culture. It was made of light-transmitting plastic (PMMA). Mixing was created by the tip speed (Eq. 2) of a spinning plate with skirt, where r is the radius or the spinning plate, and ω the angular velocity.

$$u_{Tip} = r \times \omega \quad (2)$$

While spinning, the velocity of a spinning plate with skirt created a hydrodynamic (shear) force between spinning plate and wall (Tennekes and Lumley 1972; Hosaka et al. 1995; Sánchez Pérez et al. 2006) and spatially homogeneous bottom stresses (τ). Metered fluid is recirculated simultaneously through the center axis (Fig. 1). Bottom shear stress relates to u* (friction velocity) by dividing former by the fluid density σ (Tennekes and Lumley 1972; Gust 1989):

$$u* = (\tau/\sigma)^{1/2} \quad (3)$$

The spinning plate is located 5 cm above the bottom. The turning rates (stirring speed) of the shown calibration curve for friction velocity (Fig. 2) for up to 250 revolutions per minute (rpm) were obtained by direct calibration using hot-film anemometry. Higher rates are calculated by means of an empirical model based on hydrodynamics of a boundary layer theory (Schlichting 1968). The correlation between stirring speed and TS was also calculated and shown in Fig. 2. For evaluating both forces the surfaces where the forces occur were calculated. TS with directly resulting forces occurs at a surface of 644 cm^2 at the wall and spinning plate of the microcosm and is the main (shear) force in the present study. The Reynolds stress (shear stress) in the water head between spinning plate and wall during equilibrium mean flow are comparable to the bottom shear stress and can be expressed with $u_*^2 = \overline{-u1 \times u2}$ (Tennekes and Lumley 1972). Calibrated friction velocity occurs at 314 cm^2 of the bottom of the microcosm and is the secondary, minor shear force in present study which requires for high-precision experiments to utilize known combinations of plate turning rates and metered central fluid recirculation. Since the fluid recirculation path affects only approximately one third of the inner radius, we omitted that detail at the expense of a slightly larger inhomogeneity of the averaged bottom stress. As a comparison to other studies in biotechnology, (bottom) shear stress (in Pa) can be calculated by Eq. 3, taking conversion factors from cm s^{-1} into Pa into account. In a pond, the friction velocity is approximately 4 % of the mean flow (cm s^{-1}).

The microcosm was filled with 1.75 L of microalgae culture, and the temperature of the system was maintained constant at 20 °C by the temperature control within the climate chamber. The microcosm was supplied with a mean irradiance of 122 μmol photons m^{-2} s^{-1} at a day/night cycle of 16/8 hours. The different microalgal strains show different light sensitivities; thus, their cell densities determined by optical density (OD_{750}) were adjusted to OD_{750}=1.0 for *C. vulgaris* and OD_{750}=3.0 for *S. obliquus* and *C. reinhardtii,* to avoid light stress during measurements of the effective quantum yield. The pH value and the aeration remained the same as during preculturing, unless mentioned otherwise.

Microcosm experiments

Three different types of experiments were conducted in the microcosm. In a first series of experiments, all three microalgal strains (*C. vulgaris, S. obliquus,* and *C. reinhardtii)* were cultivated in short-term experiments in the microcosm. Thereby, each algae strain was treated with different TS (0–589 cm s^{-1}) and resulting friction velocity (0 to 6.05 cm s^{-1}) which was increased stepwise. The minimum duration of each step was 3 min and the respective effective quantum yield was measured. In order to determine changes and to compare the results, the photosynthetic activity (PA) was determined. For that, the effective quantum yield of the unstirred control of each microcosm experiment (normally 0.72–0.78) was set as a control value of 100 % and each further data of the experiment were calculated relative to this value.

In a second series of experiments (long term), the increase of biomass and growth rate at the optimal TS and its friction velocity, determined by PA of the first experiment series, was investigated over 4–7 days.

A third series of experiments was similar to the first one. Changes in pH of the media (6.0–8.5) and aeration with different concentrations of CO_2 ranging from normal air (0.038 % CO_2) and CO_2-enriched air 4 % (*v*/*v*) were conducted. In the pH experiment, the unstirred Φ_{PSII} value of pH 7.0 was set to the control value of 100 % to calculate the PA. In the CO_2 experiment, the unstirred Φ_{PSII} value of the 0.038 % (*v*/*v*) CO_2 aeration was set to the control value of 100 % to calculate the PA.

Results

Photosynthetic activity at different tip speed

The influence of a defined mixing, given as TS and friction velocity on the effective quantum yield Φ_{PSII}, as a new measuring parameter, of different algae species was determined. Therefore, each microalgae strain was treated with different TS, which was increased stepwise from 0 to 589 cm s^{-1} and resulting friction velocity from 0 to 6.05 cm s^{-1} (Fig. 3). At the start of the experiment, the microalgae always exhibited a high activity and fitness, as shown by a high effective quantum yield of 0.72–0.78.

Independent from the variations in the culture conditions of *S. obliquus* and *C. vulgaris*, the photosynthetic activity

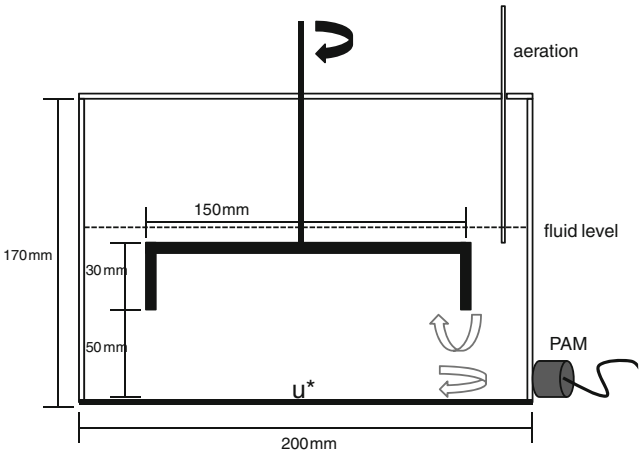

Fig. 1 Scheme of the microcosm with its spinning plate for stirring. The calibrated friction velocity acts on the bottom of the microcosm (*u**). Other surfaces where shear stress occurs are the walls and the spinning plate due to tip speed. The *arrows* indicate the stirring and flow directions. The measuring head of the PAM device was mounted on the wall next to the bottom

followed the same pattern in its dependency on tip speed, thus wall shear stress, and friction velocity (u*) (cm s^{-1}). As shown in Figs. 3 and 4, this pattern was characterized by an increase in photosynthetic activity with increasing TS, compared to an unstirred culture ($PA_{TS\ =\ 0}$) and reached a maximum in PA (PA_{max}) at an TS_{PAmax} of 126 cm s^{-1} (2.09 cm s^{-1} friction velocity). A further increase of TS beyond TS_{PAmax} resulted in a decrease in PA lower to 100 % at TS_{PA100} (further explanation of parameters see legend Table 1). Hence, at this TS, the positive effects of stirring were compensated by the negative effects. At higher TS, the negative effects dominated the positive ones and PA dropped below 100 % and reached $PA_{TS\ =\ 589}$ at the highest TS applied during the experiments. These parameters of the graph, which describe a general pattern, were used to compare the results under different culture conditions, summarized in Table 1.

Figure 3 shows that at a tip speed of 126 cm s^{-1} (friction velocity of 2.09 cm s^{-1}), the photosynthetic activity of *C. vulgaris* reached a maximum with a 4.0 % higher activity compared to the unstirred control. *S. obliquus* showed a similar increase in photosynthetic activity by 4.8 % at TS between 63 and 126 cm s^{-1}. With further increase of TS above 126 cm s^{-1}, the photosynthetic activity of both *S. obliquus* and *C. vulgaris* continuously decreased and reached PA values of 92.3 and 93.3 % at 589 cm s^{-1}, respectively. The photosynthetic activity detected at TS of 203 cm s^{-1} was similar to that of an unstirred culture; at this value, the positive effects of friction velocity were compensated by the negative effects both in *S. obliquus* and *C. vulgaris*. *C. reinhardtii* showed no positive effects by TS on photosynthetic activity, and PA of 100 % were detected at TS between 0 and 49 cm s^{-1}. A further increase in TS caused a continuous decrease in PA to 85 % at TS of 589 cm s^{-1}, whereby at TS above 203 cm s^{-1}, the decrease in PA was higher. Thus, TS of 203 cm s^{-1} can be regarded in all these algal strains as a threshold value above which the negative effects of TS became stronger than the positive effects.

The experiments at different pH revealed that changes in pH between 6 and 8.5 had no significant effect on the photosynthetic activity in the unstirred culture of *C. vulgaris*. In contrast, *S. obliquus* was negatively affected at pH 6.0 and 8.5, and photosynthetic activity decreased in the unstirred cultures ($PA_{TS\ =\ 0}$) relative to the control at pH 7.0 by 7 and 3 %, respectively (Table 1). For both algal strains, an increase in TS resulted at the different pH values in an increase in photosynthetic activity of 3 to 8 %, and a PA_{max} of 99.1–105 % was reached at a TS (TS_{PAmax}) of ≤157 cm s^{-1}. TS_{PA100} and $PA_{TS\ =\ 589}$ detected in the cultures of *C. vulgaris* and *S. obliquus* varied little at different pH values of 6.0, 7.0, and 8.5, and values were between 159 and 203 cm s^{-1} and 89.7 and 94.1 %, respectively.

The supply of CO_2 with a concentration of 4 % (*v*/*v*) resulted in an increase in photosynthetic activity in the unstirred culture of *C. vulgaris* by 5 %, compared to CO_2

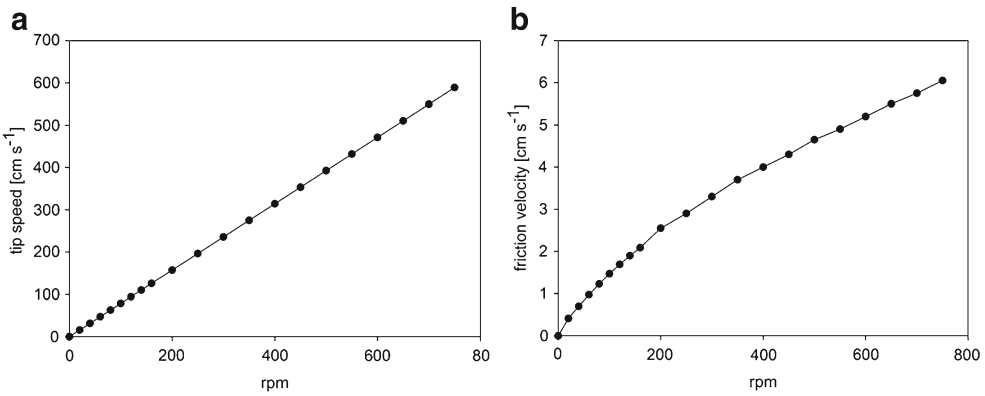

Fig. 2 Correlation between stirring speed (rpm) of the microcosm and resulting tip speed (**a**) and friction velocity u* (cm s^{-1}) (**b**)

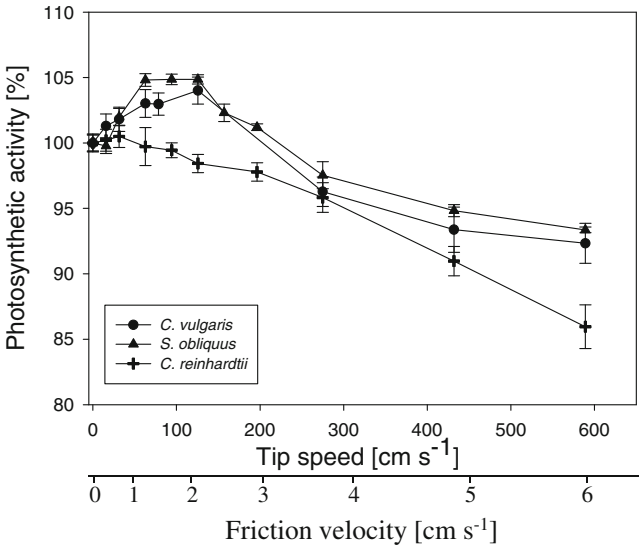

Fig. 3 Dependencies between the photosynthetic activity of *S. obliquus, C. vulgaris*, and *C. reinhardtii* and the tip speed, as well as the friction velocity. PA is given as arithmetic mean and standard deviation from continuous measurements during a period of 3–4 minutes of constant TS (n=20). TS was increased stepwise after each measuring period

of 0.038 % (*v*/*v*) (Fig. 4), while *S. obliquus* and C. *reinhardtii* remained unaffected (Table 1). An increase in PA with increasing TS was similar to the shown pH 7.0-experiment (Fig. 3), which indicated that CO_2 did not cause the increase in PA with increasing TS in *S. obliquus*. In the *C. vulgaris* culture, only a part of the increase was due to a better availability of CO_2. A 2 % increase of the photosynthetic activity with 4 % (*v*/*v*) CO_2 supply was lower than the 3 % without CO_2 supply. The effect of CO_2 addition seemed to be low compared to the effect of TS as it is indicated by the results for TS_{PA100} and $PA_{TS\ =\ 589}$ which were not significantly different from those detected under different pH values and low CO_2 concentrations. Mean values were thus calculated for TS_{PA100} and $PA_{TS\ =\ 589}$ which describe the general dependency of both *C. vulgaris* and *S. obliquus* on TS above TS_{PAmax} (Table 1).

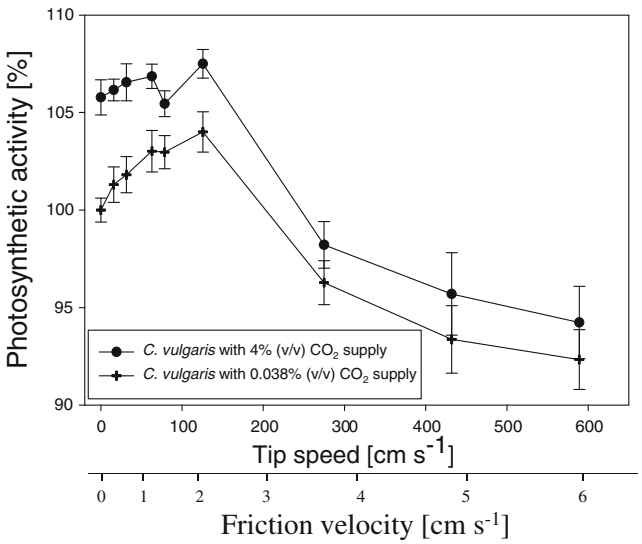

Fig. 4 Dependencies between the photosynthetic activity of *C. vulgaris* and the tip speed during aeration of CO_2-enriched air of 0.038 and 4 % CO_2 (*v*/*v*) in the culture medium. PA is given as arithmetic mean and standard deviation from continuous measurements during a period of 3–4 minutes of constant tip speed (n=20). TS was increased stepwise after each measuring period. PA of 100 % corresponds to the unstirred culture at 0.038 % CO_2

Growth at constant tip speed and friction velocity

Growth of stirred *C. vulgaris*, *S. obliquus*, *C. reinhardtii*, and *A. flos-aquae* cultures at a constant tip speed of 126 cm s^{-1} and friction velocity of 2.09 cm s^{-1} was determined in comparison to unstirred cultures (TS of 0 cm s^{-1}) (Fig. 5a–c). In the first experiment series, a tip speed of 126 cm s^{-1} was investigated as a value for the maximum photosynthetic activity of the first two mentioned microalgal species. After six days of cultivation, biomass increase in the unstirred and stirred cultures of *C. vulgaris* were 1.0 and 1.48 g L^{-1}, respectively, and thus the stirred culture showed a 48 % higher growth (Fig. 5a). The specific growth rate was 21 % higher in the stirred culture than in the unstirred one with μ_{spec} of 0.40 and 0.33 day^{-1}, respectively. While the growth rate of the stirred culture seems to be constantly high, the slope of the graph of the unstirred culture decreases at the end of the experiment. This could be interpreted as light limitation of cells in shadow. A positive effect of TS on growth was also found for *S. obliquus*, and the specific growth rate of the stirred culture was 35 % higher than of the unstirred one. μ_{spec} was 0.27 and 0.20 day^{-1}, respectively, corresponding to an increase of biomass during the four days of cultivation of 0.77 and 0.45 g L^{-1}, respectively (Fig. 5b).

C. reinhardtii which showed no positive effects of TS and friction velocity on photosynthetic activity (Fig. 3) also exhibited finally a 7 % higher specific growth rate during 7 days of cultivation in the stirred compared to the unstirred culture, with μ_{spec} of 0.29 and 0.27 day^{-1} and a biomass increase of 0.84 and 0.72 g L^{-1}, respectively (Fig. 5c). Photosynthetic activity was 2 % lower in the stirred culture of *C. reinhardtii* than in the unstirred culture; thus, the faster growth under stirring was not reflected by this parameter and must have another reason.

Growth experiments with *A. flos-aquae* revealed that this filamentous cyanobacterium was lethally damaged by stirring which generated TS of 126 cm s^{-1} after 2 days of cultivation. Biomass decreased from 0.22 to 0.14 g L^{-1}. Microscopic analysis at the end of the

Table 1 Influence of pH values and different CO_2 concentrations on all three microalgae strains revealed at special parameters of the graph

	$PA_{TS=0}$ (%)	PA max (%)	TS_{PAmax} (cm s^{-1})	$PA_{TS=589}$ (%)	TS_{PA100} (cm s^{-1})
pH					
C. vulgaris					
7	100.0	104.0	126	92.3	203
6	100.7	105.0	63	89.7	188
8.5	98.8	102.0	126	93.5	188
S. obliquus					
7	100.0	104.8	126	93.3	159
6	92.3	100.4	157	94.2	159
8.5	97.4	99.1	157	94.1	–
C. reinhardtii					
7	100.0	100.5	31	85.0	49
CO_2 conc. (*v*/*v*)					
C. vulgaris					
4 %	105.0	107.5	126	95.0	236
0.038 %	100.0	104.0	126	93.0	196
S. obliquus					
4 %	99.2	104.0	126	92.5	196
0.038 %	100.0	102.5	126	97.5	345
C. reinhardtii					
4 %	100.0	100.5	31	85.0	49
0.038 %	100.0	100.	0	80.0	0

$PA_{TS=0}$ PA in the unstirred control, $PA_{max}(\%)$ Maximum PA, $TS_{PA\ max}$ tip speed at maximum PA, $PA_{Ts=589}$ PA at maximum tip speed of 589 cm s^{-1}, TS_{PA100} tip speed where PA reaches the value of the unstirred control

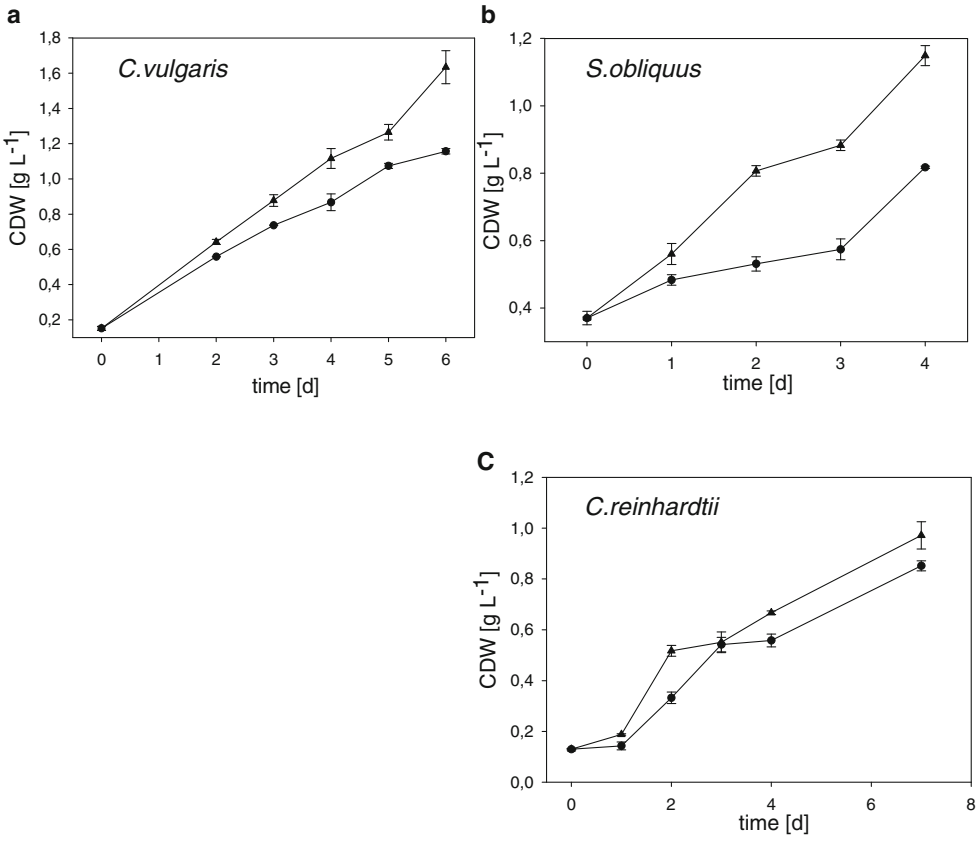

Fig. 5 Increase in biomass of three microalgal strains during 4–7 days of cultivation under stirred (*triangles*) (TS= 126 cm s^{-1}) and unstirred conditions (*circles*). Means with standard deviation of CDW triplicates. **a** *C. vulgaris*; **b** *S. obliquus*; **c** *C. reinhardtii*

experiment revealed that the filaments of *A. flos-aquae* were destroyed and disintegrated into segments of 1–4 cells (data not shown).

Discussion

The effect of a defined mixing and shear forces affecting photosynthesis measured by PAM technology is a new methodology. In most studies described in the literature so far, the effect of shear forces on microalgae was determined by changes in growth and viability of the organism (Contreras et al. 1998; Jaouen et al. 1999; Michels et al. 2010). Growth depends on the sum of different metabolic activities; thus, the cause–effect relationships underlying the effect of hydrodynamic forces was so far little elucidated, because this requires a detection method which resolves changes on the time scale of minutes. Hosaka et al. (1995) showed a response of hydrodynamics by measuring oxygen production of microalgae. The photosynthetic system is a good indicator system because it is both very sensitive to any changes in the living conditions. To overcome restrictions, PAM fluorescence yield reflecting the photosynthetic activity of different microalgae was used in this study to detect the effect of mixing in case of tip speed and friction velocity. It is favorable, because it is known to respond within seconds to changes in the cultivation conditions. The fluorescence yield and the corresponding photosynthetic activity is thus an established method to study the influence of hydrodynamic forces and was successfully applied previously to describe friction and flow effects in corals and other higher marine organisms (Koch 1994; Finelli et al. 2006).

Effect of tip speed and friction velocity on photosynthetic activity

Even small changes in the tip speed and dependent friction velocity resulted in changes in photosynthetic activity, measured as effective quantum yield, of freshwater microalgae within minutes. A specific pattern reflecting the relationship between hydrodynamic forces and photosynthetic activity was thereby observed for all of the tested microalgae. For a better comparability the shear stress regarding different selected stirring speeds was calculated. Comparing tip speed with resulting hydrodynamic forces and friction velocity, tip speed is the main influencing force due to affecting a higher surface und higher velocity. In case of *C. vulgaris* and *S. obliquus*, the pattern was marked by a rapid increase in PA with increasing TS up to a maximum of PA at a TS of about 126 cm s^{-1} (u* of 2.09 cm s^{-1}, equivalent to 0.45 Pa). The subsequent decrease of PA at 203 cm s^{-1} (u* of 3 cm s^{-1}, equivalent to 0.9 Pa) indicates the state were positive effects compensated negative effects of TS. At higher tip speed, the PA further decreased and indicates lower activities than in an unstirred culture. From the similarity in the response of PA, it can be concluded that *C. vulgaris* and *S. obliquus* might have a similar physiological response to hydrodynamic forces and that the underlying causalities are the same. This conclusion is supported by the finding that both microalgae showed about the same increase in PA of 4 and 4.8 %, respectively, at the optimum TS (126 cm s^{-1}) and friction velocity of 2.09 cm s^{-1}. Compared to sea grass, it was shown that increasing friction velocity in a microcosm resulted in an increase in oxygen production with an optimum between 0.5 and 0.8 cm s^{-1} (Koch 1994).

In *C. reinhardtii*, the photosynthetic yield clearly decreased up to 2.3 % due to stirring and friction velocity of 2.09 cm s^{-1}, while the growth slightly increased. The pattern of the *C. reinhardtii* response is conspicuous due to rising TS. It is a motile unicellular organism, with a protein-containing soft cell wall and a flagellum and, thus, morphologically more sensitive to tip speed and friction velocity. A positive physiological response was not detectable on the level of photosynthetic activity but only on growth as discussed below (Table 2). During exposure to a TS of 589 cm s^{-1} (u* of 6.05 cm s^{-1}, which is equivalent to 3.66 Pa), the PA decreased in *C. vulgaris* and *S. obliquus* by about 8 % and in *C. reinhardtii* by about 15 % compared to the unstirred control. When cultures were subsequently exposed to unstirred conditions, the PA recovered to 100 % within minutes (data not shown). This finding is in accordance with previous studies which showed that microalgae can adapt to shear stress (Hosaka et al. 1995) and their physiological characteristics recover even after exposition to high free-jet shear stresses of up to 10^7 Pa (Bronnenmeier and Märkl 1982). Compared to the study of Hosaka et al (1995) and Mitsuhashi et al (1995), where short term shear stress (20 minutes) caused a decrease in oxygen production and long-term shear stress (6 days), an increased growth, both PA (short term) and growth (long term) in *C. vulgaris*, were enhanced by shear stress in terms of a certain friction velocity in this study. In contrast, oxygen production decreased linearly due to a shear stress of 0–0.4 Pa to ≥50 %, depending on the culture temperature. No short-term optimum in shear stress was found (Hosaka et al. 1995).

Table 2 Enhanced photosynthetic activity, growth rate, and biomass production of different microalgae during tip speed of 126 cm s^{-1} compared to control of 0 cm s^{-1}

Organism	PA (% of control)	Growth rate (% of control)	Biomass production (% of control)
C. vulgaris	+4.0	+21	+48
S. obliquus	+4.8	+35	+71
C. reinhardtii	−2.3	+7	+14

Effect of tip speed and friction velocity on growth and morphology

Photosynthesis delivers energy for growth in photoautotroph organisms. The rise in PA with hydrodynamic forces was expected to be directly related to an increase in algal growth. Our findings, however, show that the enhanced growth rates and biomass production studied at the optimum tip speed of 126 cm s^{-1} reached 21–35 % and 48–71 % in *C. vulgaris* and *S. obliquus*, respectively, a 10- to 15-fold stronger increase than in PA (Table 2). The observed finding was that the PA does not reflect the whole positive effect of the mixing and resulting friction velocity can be explained by that growth includes most of the physiological processes within a cell which can all be enhanced by hydrodynamic forces, whereas the yield (Φ_{PSII}) only displays energy transfer rates within the photosynthetic system. It is important to note that PA was measured from the cells in the moment closed to the wall of the microcosm, whereas the growth displays the effect on the whole culture. This has constructive reasons as the induced fluorescence pulse only from irradiated cells close to the microcosm wall will reach the detector in time. Photosynthetic activity depends on irradiance and light/dark frequencies. Mixing due to tip speed can cause an improved light exposure corresponding to each single cell (Sobczuk et al. 2006). This occurs especially under high light conditions above light saturation, when cells can oscillate between different light layers. Hence, it can be expected that during cultivation at light intensities below light saturation, the photosynthetic yield increases slightly with TS due to a better light regime. For this reason, other factors in addition to light must have caused the positive effect of TS on PA as well. Mass and gas transfer especially in mass cultures are optimized by mixing in case of TS (Contreras et al. 1998), and cell division process is enhanced by shear forces (Chisti 2001). Furthermore, mixing is necessary to maintain a constant, homogenous pH (Kommareddy and Anderson 2005) and temperature (Oswald 1988) and to prevent sedimentation. Thus, any of these effects might have caused the enhanced growth rate and biomass production of *C. reinhardtii*, *C. vulgaris*, and *S. obliquus* between 7–35 and 14–71 %, respectively, when TS was increased to 126 cm s^{-1} (u* of 2.09 cm s^{-1}, equivalent to a shear stress of 0.45 Pa) (Table 2).

Concerning shear stress, Hosaka et al. (1995) found that growth was enhanced with rising shear stress, and the. highest applied shear stress of 0.6 Pa over 6 days resulted in 50 % higher growth in *C. vulgaris*. Our results for calibrated friction velocity are in accordance, 0.45 Pa resulted in a 48 % better growth. For *S. obliquus*, the growth was even higher. The difference in growth compared to Hosaka et al. (1995), can be explained by different light conditions, temperature, or composition of the culture medium in the experiments. Contreras et al. (1998) determined with *Phaeodactylum tricornutum* in a concentric tube airlift PBR growth rates due to shear rates. Growth was enhanced from a shear rate of 3×10^{-3} s^{-1} to an optimum of 7×10^{-3} s^{-1} up to 53 %. Above shear rates of 7×10^{-3} to 14×10^{-3} s^{-1}, the growth rate decreased by 26 %. This is equivalent to shear stresses of 3×10^{-6} to 14×10^{-6} Pa, assuming a viscosity of about 1 mPa*s (Lorch, University of Hamburg, unpublished data). The finding that an optimum for shear stress exists is in accordance with our pattern detected for the response of photosynthetic activity and growth to friction velocity. The optimal friction velocity found for *P. tricornutum* is, however, much lower than the 2.09 cm s^{-1} as for our *C. vulgaris* and *S. obliquus* experiments, as diatoms seemed to be more sensitive. For *C. reinhardtii*, we also found that a rise in friction velocity from 0 to 2.09 cm s^{-1} resulted in a much lower increase in biomass and growth rate of 14 and 7 %, respectively, than in *C. vulgaris* and *S. obliquus*. Friction velocity means a significant stress on algae which are morphologically more sensitive, especially flagellates (Jaouen et al. 1999). To obtain the benefits of mixing but to lower the effects of resulting shear forces, protective substances, for example, carboxymethyl cellulose, can be added into the culture medium of airlift PBRs (Mirón et al. 2003). *A. flos-aquae*, a filamentous cyanobacterium was clearly damaged by shear forces. It is possible that primarily, the friction velocity supports growth, but with longer exposure time, the filaments might have been damaged and did not grow anymore (Thomas and Gibson 1990). Comparing the obtained shear stress to the current results from other researchers, where cells get lethally damaged, in our microcosm, much lower shear stress was obtained; a maximum of 0.27 Pa compared to 10 Pa (Michels et al. 2010). This shear stress killed the diatom *C. muelleri* because it seems that organisms with silicate cell wall are more sensitive (Michels et al. 2010). The difference in sensitivity to friction velocity of the algae in the study described above is in accordance with those described in literature for pressure. Free-jet experiments revealed that *Chlorella* can deal with and recover from 10^7 Pa of free-jet pressure, whereas *C. reinhardtii* only recovered from 1.5×10^6 Pa and *Spirulina platensis* (filamentous, comparable to *A. flos-aquae*) only from 2×10^5 Pa (Bronnenmeier and Märkl 1982). This accordance in sensitivity further strengthens our conclusion that the positive physiological effects of friction velocity occur the same way in all algae, but these are counterbalanced by the negative effects of the morphological stress which depends on the sensitivity of the cell wall and other morphological structures to hydrodynamic forces. Of course, free-jet pressure differs very much from friction velocity, but interestingly, the effect on the cells is in the same order.

Cause–effect relationship of tip speed

To find a cause–effect relationship of the increasing PA with tip speed, experiments were done under different pH values,

CO_2 concentrations, and nutrient content (data not shown). Variations in pH between 6.0 and 8.5 and nutrients did not have an influence on the effect of TS on PA, which does not mean these factors had no effect on PA. The photosynthetic apparatus did not respond fast enough to reflect nutrient uptake which was most likely both enhanced by an increase in nutrients and lower pH value. Lippemeier et al. (2001) found that fluctuating nutrient supply and its recovery can be recorded with PAM fluorescence technology only when nutrients are added which directly enhance photosynthesis or its enzymes or metabolites. This was not the case in our experiments where nutrient concentrations were always above limitation (Lippemeier et al. 2001).

In contrast, increasing CO_2 concentrations (4 % *v*/*v*) clearly increased PA in all microalgae. This is in accordance with the findings in plants which exhibit higher photosynthetic yield when CO_2 supply was improved (Björkman and Demmig 1987). However, the increase of PA by adding CO_2 was more or less similar and independent from the TS applied in our experiment. The relationship between TS> 126 cm $s^{-1}$and PA remained constant at different CO_2 concentrations; the observed increase in PA with TS was not due to a better supply of CO_2. A slight positive effect may occur only at lower TS (Fig. 4).

Because pH (+ nutrients) and CO_2 supply were obviously not the ruling main factors which enhanced physiological processes at increasing tip speed, it can be also assumed that hydrodynamic forces (mixing due to tip speed and friction velocity) directly favored intracellular processes. Previous studies showed that temperature-dependent shear stress affects mechanical properties and physiological structure of the cell membrane (Mitsuhashi et al. 1995). It also influences membrane permeability and metabolite production rate (Merchuk 1991). In addition, TS and shear stress produce small-scale differences in pressure and could thus increase intracellular transport processes. Further studies are needed to find out if intracellular effects occur and to what extent they cause an increase in metabolic activities.

In conclusion, the results from all four studied algae suggest that each alga or taxonomic group shows a different response to hydrodynamic stimuli. In the present study, it was shown that tip speed and friction velocity has both a positive effect on metabolic activities and a negative effect due to morphological stress. The morphologically robust species *C. vulgaris* and *S. obliquus* benefit more from the positive effects of both forces because negative effects are less expressed and occur only at higher tip speed. Even morphologically sensitive species benefit from tip speed and shear forces as long as the positive effects are not compensated by the morphological stress or even cell damage. It can be summarized that each algal species has its own hydrodynamical growth optimum and that has to be taken under consideration when cultivating microalgae and designing production systems. So the best cultivation system for the desired microalgae strain has to be chosen, regarding the flow velocity (tip speed) and shear forces caused by pumps, flow, airlift systems, or the PBR geometry itself. This can be tested first, in hydrodynamically calibrated systems, like the microcosm, where defined shear forces can be applied.

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Chapter 4 Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors

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The author of this dissertation conducted all preliminary and final experiments including the preliminary tests of the pilot plant operation, invented the “temperature mode” for temperature regulation and the “offset mode” for the reduction of photoinhibition. Furthermore the data were analyzed and prepared for the manuscript. Together with the coauthors the manuscript were finished for publication.

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ORIGINAL PAPER

Irradiance optimization of outdoor microalgal cultures using solar tracked photobioreactors

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Abstract Photosynthetic activity and temperature regulation of microalgal cultures (*Chlorella vulgaris* and *Scenedesmus obliquus*) under different irradiances controlled by a solar tracker and different cell densities were studied in outdoor flat panel photobioreactors. An automated process control unit regulated light and temperature as well as pH value and nutrient concentration in the culture medium. CO_2 was supplied using flue gas from an attached combined block heat and power station. Photosynthetic activity was determined by pulse amplitude modulation fluorometry. Compared to the horizontal irradiance of 55 mol photons m^{-2} d^{-1} on a clear day, the solar tracked photobioreactors enabled a decrease and increase in the overall light absorption from 19 mol photons m^{-2} d^{-1} (by rotation out of direct irradiance) to 79 mol photons m^{-2} d^{-1} (following the position of the sun). At biomass concentrations below 1.1 g cell dry weight (CDW) L^{-1}, photoinhibition of about 35 % occurred at irradiances of ≥1,000 µmol photons m^{-2} s^{-1} photosynthetic active radiation (PAR). Using solar tracked photobioreactors, photoinhibition can be reduced and at optimum biomass concentration (≥2.3 g CDW L^{-1}), the culture was irradiated up to 2,000 µmol photons m^{-2} s^{-1} to overcome light limitation with biomass yields of 0.7 g CDW mol photons^{-1} and high photosynthetic activities indicated by an effective quantum yield of 0.68 and a maximum quantum yield of 0.80 (F_v/F_m). Overheating due to high irradiance was avoided by turning the PBR out of the sun or using a cooling system, which maintained the temperature close to the species-specific temperature optima.

Keywords Solar tracked photobioreactors · Photoinhibition · Outdoor cultivation · Microalgae · Temperature and irradiance control · Flue gas

Abbreviations

CDW	Biomass concentration, cell dry weight [g L^{-1}]
$\Delta F/F'_m$	Effective quantum yield of photosynthesis
F_v/F_m	Maximum quantum yield of photosystem (PS) II
OLP	Optical light path [mm]
PAR	Photosynthetic active radiation (µmol photons m^{-2} s^{-1}, 400–700 nm); horizontal PAR (PAR absorbed by a horizontal plane)
PBR	Photobioreactor
PS II	Photosystem II

Introduction

The demand of microalgal biomass as a possible source of fine and bulk chemicals, as a food or feed additive, as well as for renewable energy caused an increase in research and development in microalgae biotechnology during the last years [1–6]. Generally, microalgal production systems are independent of agricultural land and, therefore, do not compete with food production as proposed in the recent discussion of "food or fuel" for renewable resources [7, 8]. At present, two main concepts exist for cultivating

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microalgae: open ponds and closed photobioreactors (PBRs) [9, 10].

Outdoors, mainly pond systems, are economically successful. For an outdoor cultivation in northern temperate climates like in Germany, open ponds are difficult to operate, because of the unfavorable, fickle climatic conditions, e.g. rain and low temperature. Thus, closed systems are needed for such applications [11]. With the application of PBRs, we are faced to some problems, compared to open ponds. On the one hand, overheating of the culture due to strong sunlight occurs, especially in PBRs with high surface to volume ratios. On the other hand, higher total costs are expected [12]. In addition to the static PBR systems, as well as in open ponds, no irradiance regulation can be applied.

A suboptimal culture temperature decreases the growth rate of microalgae and overheating above a critical temperature results in culture collapse [13]. Thus, temperature regulation is necessary and commonly realized via water evaporation in outdoor systems [11]. Regarding the regulation of irradiance, however, there is a trade-off between maximum light use and light stress in microalgal cultures. During natural diurnal cycles, periods of high solar irradiances occur that are generally above the light saturation of photosynthesis [9]. This can cause photoinhibition of photosynthesis [14] and decreases energy utilization of photosynthesis for biomass production in microalgal cultures [15]. This occurs especially during cultivation at low cell densities where self-shading does not prevent photoinhibition. Longer periods of strong light stress may even cause photo-bleaching of cells, resulting also in a collapse of the culture [16]. Strong mixing in combination with a short light path results in an intermittent light regime of short light/dark cycles to which the microalgal cells are exposed between surface and deep layers, especially when cell densities are high. A high cell density culture can be exposed to high irradiance without causing photoinhibition due to the self-shading effect [17–19]. Other possibilities to prevent photoinhibition outdoors are a reduction of photosynthetic active radiation (PAR) by artificial shading or by variations in orientation and distance between installed PBR rows [14, 20]. Contrarily under light limitation, an increase in irradiance results in higher biomass productivity [21]. Thus, the aim of this work was to study the advantages of solar tracked PBRs to solve the above-addressed problems:

1. The possibility to decrease photoinhibition of photosynthesis in a microalgal culture of low density, by reducing the irradiance.
2. Enhancing the irradiance beyond 100 % of the horizontal irradiance in high cell density cultures by exposure of the reactor perpendicular to the sun light.
3. Regulating culture temperature by adjusting the irradiance or cooling to avoid heat stress.

Fig. 1 Flat panel photobioreactors mounted on a solar tracker. The optical light path of the PBRs varied between 15 and 22 mm

All experiments were performed at a prototype outdoor pilot plant situated in Northern Germany using natural sunlight and flue gas from a power plant as energy and carbon sources, respectively. The microalgal species *Chlorella vulgaris* and *Scenedesmus obliquus* were used, which are common for outdoor mass cultivation [22, 23]. Both strains are flue gas tolerant, and mass cultures can be adapted to high irradiance of direct sunlight.

Materials and methods

The photobioreactor system

The microalgae pilot plant is located in Hamburg-Reitbrook in Northern Germany (53°28′12″N; 10°10′40″E) (Fig. 1). The outdoor PBRs used in this study (European patent No. 2228432, developed by SSC Ltd.) were mounted on two turnable towers, so-called solar trackers, normally used as standard technique to track photovoltaic panels (ST2000, SolarTrack Anlagenbau Ltd., Germany). In the standard orientation, the trackers followed the position of the sun, so that maximum irradiation was always obtained with sunlight at a 90° angle to the PBR surface. This includes horizontal and vertical variations in the azimuth and altitude. In a special parallel orientation ("offset orientation", 0° angle to position of the sun), the trackers rotated out of direct sunlight and the PBRs were exposed from the narrow side only. This orientation was used both, to obtain reduction of irradiance on the PBR surface and to prevent overheating of the culture medium.

Up to seven PBR panels were mounted on one solar tracker, each covering 2 m^2 and containing 30–45 L of microalgal suspensions with an optical light path (OLP) of

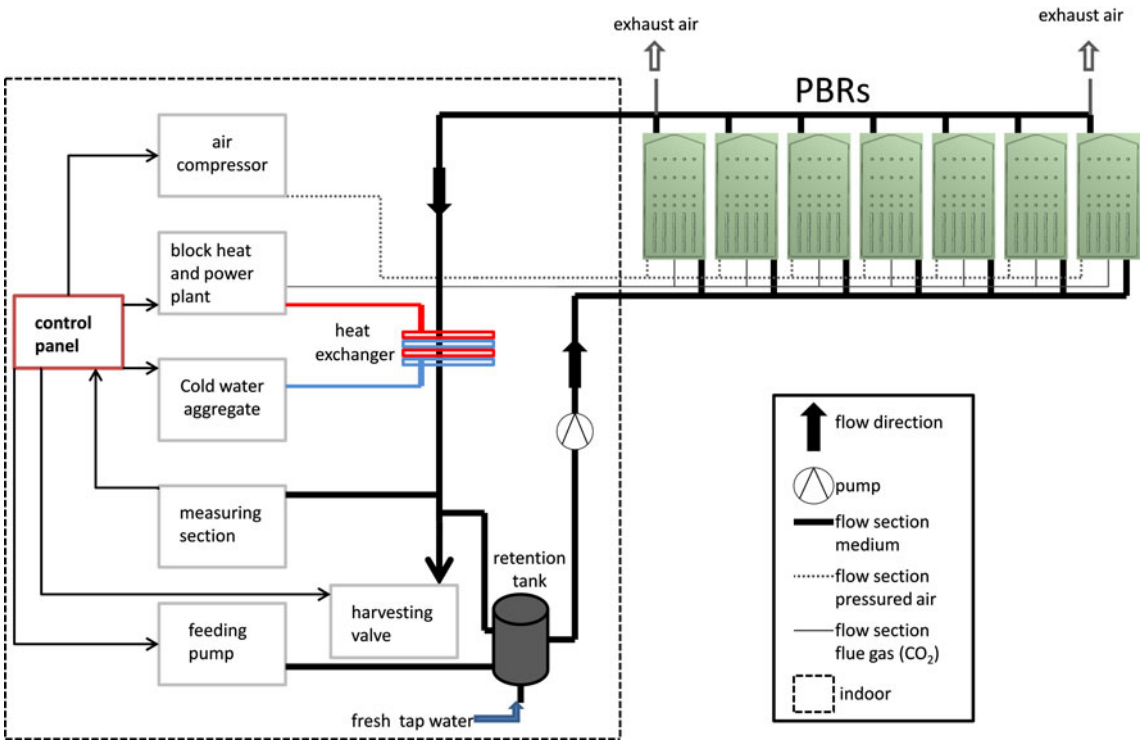

Fig. 2 Flow chart of the function of the microalgae pilot plant. During cultivation, part of the culture medium circulates through an external circuit. This allows to harvest algal biomass continuously and, thereby, adjusts cell density to a constant value (turbidostat mode). The process parameters were monitored by an online measurement of temperature, O_2, pH, turbidity, NH_4^+, NO_3^-, and K^+. Nutrients were added automatically and, thus, kept above a set value. A heat exchanger within the circular flow allowed temperature control (heating and cooling). The pumps for establishing medium flow, the sensors, a process control panel, a block heat and power plant, an air compressor, and heat exchangers were located indoor (marked by a *dashed line*)

15–22 mm. Including the tube volume (30–35 L) for circulation and culture control, the maximum total volume per solar tracker was 240 L and the surface [m^2] to volume [m^3] ratio of the PBRs was 66 m^{-1}. The culture medium circulated with 1 L PBR^{-1} min^{-1} in the PBR system. The flow cycle included beside the PBRs, a measuring section and a retention tank (10 L) where tap water, nutrients, and preculture were added (Fig. 2). Compressed air was periodically injected at the bottom of each PBR through valves (0.25 s^{-1} frequency, 0.4 L mean air flow L^{-1} culture medium min^{-1}) which mixed the culture. This ensured that a single cell was only shortly exposed to high irradiances close to the reactor surface. Furthermore, this decreased the biofouling on the PBR surface. Parallel to the compressed air, flue gas obtained from a combined block heat and power station was injected through a porous tube (PS 5 µm Genpore, USA) at the bottom of the PBR.

Solar track technology

The irradiance on the surface of each PBR was controlled by the solar tracker by positioning it at two different orientations to the sun. To obtain maximum irradiance, the PBRs were always exposed perpendicular to the sun rays (standard orientation). A minimum exposure was achieved by turning the PBR at a 90° angle out of the direct sun rays (offset orientation) (Fig. 3). The offset orientation was chosen with dilute culture at the start of cultivation (preculture). In contrast, maximum irradiance during standard orientation was used for cultivations at high cell densities. Due to the two orientations, the cultures could be run automatically at three different operation modes:

1. offset mode (permanent offset orientation, minimum light)
2. standard mode (permanent standard orientation, maximum light)
3. temperature mode (temperature of the culture controlled the angle to the sun).

In the temperature mode, the solar trackers turned away from the sun automatically (offset orientation) when the temperature of the culture medium increased above a certain temperature set value. If the culture temperature was below the temperature set value, the PBRs turned back into the standard orientation. For comparison, the culture temperature control was also realized in the standard mode via a cooling system, consisting of a heat exchanger and a cold water aggregate.

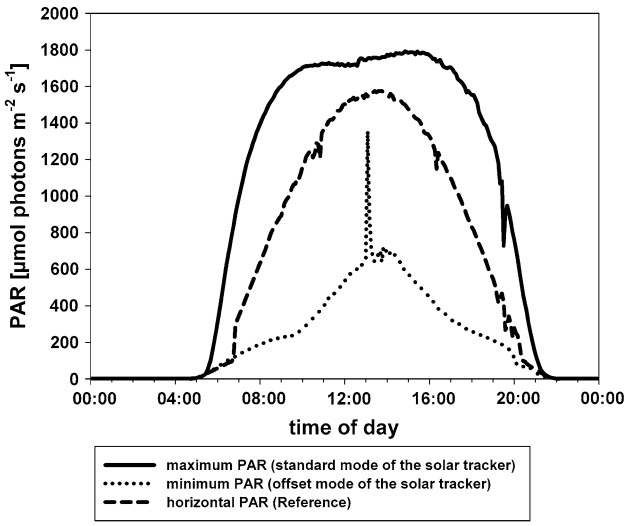

Fig. 3 Daily variations in irradiance on the PBRs mounted on a solar tracker and exposed at a constant perpendicular orientation to the sun (standard mode) or in a 180° angle to the sun (offset mode) in comparison to a horizontal plane during a clear summer day in Hamburg (Germany). The brief light peak determined in the offset mode is due to the backward shift of the tracker by 180° angle

To demonstrate the possible light regulation by the solar tracker, the impinging light and as reference the measured horizontal solar irradiance are shown in Fig. 3. The brief light peak during midday in the offset mode is related due to a 180° angular shift of the tower through full sunlight (~3 min), since a rotation of the towers over 360° angle was technically impossible.

Strains, preculture and culturing conditions

The microalga putative *Scenedesmus obliquus* (*Chlorophyta*), was isolated from the pilot plant culture, characterized by 18SrRNA sequencing and deposited in the SVCK algal collection at the University of Hamburg (No. U169). The microalga *Chlorella vulgaris* (*Chlorophyta*) was obtained from the SVCK collection (No. U126). To obtain precultures, the strains were grown in 10-L Schott flasks filled with 8 L medium using 2 g L^{-1} "Flory Basic Fertilizer 1" (Euflor, Germany) and 3.22 g L^{-1} KNO_3. After 10 days of laboratory cultivation at 20 °C, 100 µmol photons m^{-2} s^{-1} and aeration with CO_2-enriched air (4 % v/v), the culture was used to inoculate the PBR system to a starting cell dry weight (CDW) of 0.1 g L^{-1}. The PBR system was cleaned with 3–10 % H_2O_2 before each cultivation process or after an exchange of the algal species. The cleaning solution was circulated in the PBR system for 48 h and the whole system was washed twice with tap water before re-inoculation. The culture temperature in the PBR system was always kept below 35 °C using two independent techniques, a cold water aggregate while operating in the standard mode or using the solar tracker in the temperature mode. Waste heat produced by the block heat and power plant was used to keep the culture temperature above 15 °C during cold seasons.

The pH value was maintained between 6.5 and 7.5 by a combination of flue gas injections and titration of hydroxide. The upper pH set value (7.5) was established by varying the flue gas injection. The lower pH set value (6.5) was controlled by automatic titration with 5 N NaOH of the culture medium. Flue gas was obtained from a combined block heat and power station by running with natural gas. It was compressed to 150 kPa and injected into the PBRs. The composition of the flue gas is shown in Table 1.

The oxygen concentration in the PBRs ranged between 4 and 18 mg L^{-1}. Nutrient supply was controlled by measuring nitrate concentration. If the nitrate value decreased below a set value of 100 mg NO_3^--N, then the feeding media containing 60 g L^{-1} "Flory Basic Fertilizer 1" and 58.4 g L^{-1} NH_4NO_3 was automatically pumped into the culture (Chem-ad VPP-E, Siemens, Germany). All experiments were conducted in (fed-)batch modes, started with axenic cultures and operated non-aseptically.

Sensors and analytics

The cultivation conditions were monitored using industrial sensors (WTW IQ Sensor Net, System 2020 XT, Germany) which determined the concentration of NO_3^-, O_2, as well as pH, temperature and turbidity inline. The irradiance impinging onto the PBR surface was determined by a LI-190 sensor (LI-COR, USA) mounted on the tracker. The data were displayed on a control panel, which was programmed to control the culturing process. To check the accuracy of the sensors, they were verified and calibrated using special analysis kits (WTW, Germany) and standards (WTW, Germany).

Cell density, dry weight determination and biomass yield on light energy

Cell growth was monitored by measuring the optical density at 750 nm (OD_{750}). The cell dry weight (CDW) was determined gravimetrically in pre-dried 50 mL tubes. The 50 mL culture broth was centrifuged 30 min at 6,000×*g*, the supernatant was discharged and the pellet was dried (85 °C). The CDW in g L^{-1} was calculated by comparing the difference in weight between the pre-dried tube and the tube with dried pellet. Biomass yield (Y_x) on light energy (PAR) is given by equation (1) as an indicator for the photosynthetic efficiency [24]. The areal productivity (P_{area}) was determined for the time period (t) and the area [m^2] of irradiated PBR surface.

Table 1 The composition of flue gas from a combined block heat and power station, burned with natural gas and used in the present study to supply microalgae cultures with CO_2

O_2 (vol%)	CO (ppm)	Temp. (°C)	CO_2 (vol%)	Nitrogen monoxide (NO) (ppm)	Nitrogen dioxide (NO_2) (ppm)
7.2	136	12	7.6	124	25

$$Y_X = \frac{P_{area}[gm^{-2}t^{-1}]}{\text{PAR photons } [\text{mol } m^{-2}t^{-1}]}. \quad (1)$$

Photosynthetic activity and photoinhibition

Photosynthetic activity of the microalgal cultures was measured using pulse amplitude modulation (PAM) fluorometry (Dual PAM 100, Walz, Germany). The maximum quantum yield (F_v/F_m) of photosystem II (PS II) was measured after 5 min of dark adaptation in a photometer cuvette using 3 mL of outdoor culture, at a constant temperature of 25 °C with a maximum saturation pulse of ~20,000 µmol photons m^{-2} s^{-1} and a pulse length of 300 ms. A decrease in the maximum F_v/F_m is indicative for photoinhibition [25, 26]. The degree of photoinhibition was calculated by the following equation (2), while the F_v/F_m values during the night (23:00–05:00 h) were taken as completely recovered cell [27]:

$$\text{Photoinhibition } [\%] = 100 - 100\left(\frac{F_v/F_m \text{of actual sample[5 min dark adaptation]}}{F_v/F_m \text{of completely recovered cells [maximum value]}}\right) \quad (2)$$

In situ determination of the photosynthetic activity of the culture within the PBR was done by online fluorescence measurements on the surface of the PBR with the saturation pulse method and measuring light at high frequency of 400 kHz for the detection of the minimum and maximum fluorescence (F'_0, F'_m). From these values, $\Delta F/F'_m$ is calculated. F'_m represent the maximum fluorescence of light acclimated photosynthesis. To allow in situ measurements, the detector of the Dual PAM 100 was placed at the lower part of the PBR where highest mixing of the culture medium occurred. The detector reduced the irradiance on the PBR surface by 10 cm × 10 cm. Thus, the determined effective quantum yield did not directly represent full light acclimation state [slightly reduced photochemical quenching (qE)] of the cells within the irradiated area of the PBR, because a short acclimation to lower irradiance of <1 s occurred, depending on the mixing rate. The in situ measurements of the effective quantum yield were performed with *S. obliquus* biomass concentration between 7.5 and 0.5 g L^{-1}. Defined concentrations were adjusted by a stepwise dilution of the culture medium in a PBR which was subsequently exposed both to maximum light intensities (standard mode) and minimum light intensities (offset mode). Measurements were made every 10 s and arithmetic means were calculated for 5–10 min intervals.

Results and discussion

Reduction of photoinhibition

The short light path (15–22 mm) and the direct exposure of the PBRs to sunlight by the solar track system increased the occurrence of oversaturation of photosystem II. To assess the degree of photoinhibition of *C. vulgaris* and *S. obliquus* cultures, outdoor fed batch experiments with increasing biomass concentrations were performed. The results of the degree of photoinhibition (as estimated from the decrease of F_v/F_m) of a *Chlorella vulgaris* culture at low cell densities are presented in Fig. 4. F_v/F_m decreased when the irradiance increased above 1,000 µmol m^{-2} s^{-1} at cell densities between 0.2 and 1.0 g CDW L^{-1}. In the course of a 28-h experiment, photoinhibition occurred several times to the same extent, e.g. F_v/F_m values of 0.49 (8 h; 17:00) and 0.46 (26 h; 11:00) were indicative for a degree of photoinhibition of 30 and 35 %, respectively. At certain times, the degree of photoinhibition was similar both at 0.4 and 1.0 g CDW L^{-1}; however, at the higher CDW under high irradiance, substantial photoinhibition occurred with a 3-h delay. In accordance to this, the biomass yield per light integral was also similar, with 0.4 for the first day and 0.5 for the second day. The results further showed that irradiances below 1,000 µmol photons m^{-2} s^{-1} resulted in a recovery of the photosynthetic activity within hours indicated by an increase in the values of F_v/F_m to about 0.7 (22 h). The decrease in photosynthetic activity of about 35 % exhibited a heat dissipation of sun energy, which indicates that the culture adapted to the fluctuating light regime by acclimation of its photosynthetic apparatus (27 h, Fig. 4). In literature, photosynthetically well-performing cultures may show midday depression of F_v/F_m of 20–30 %, as the depression of F_v/F_m indicates low-light acclimated or photoinhibited cultures [28]. There, a

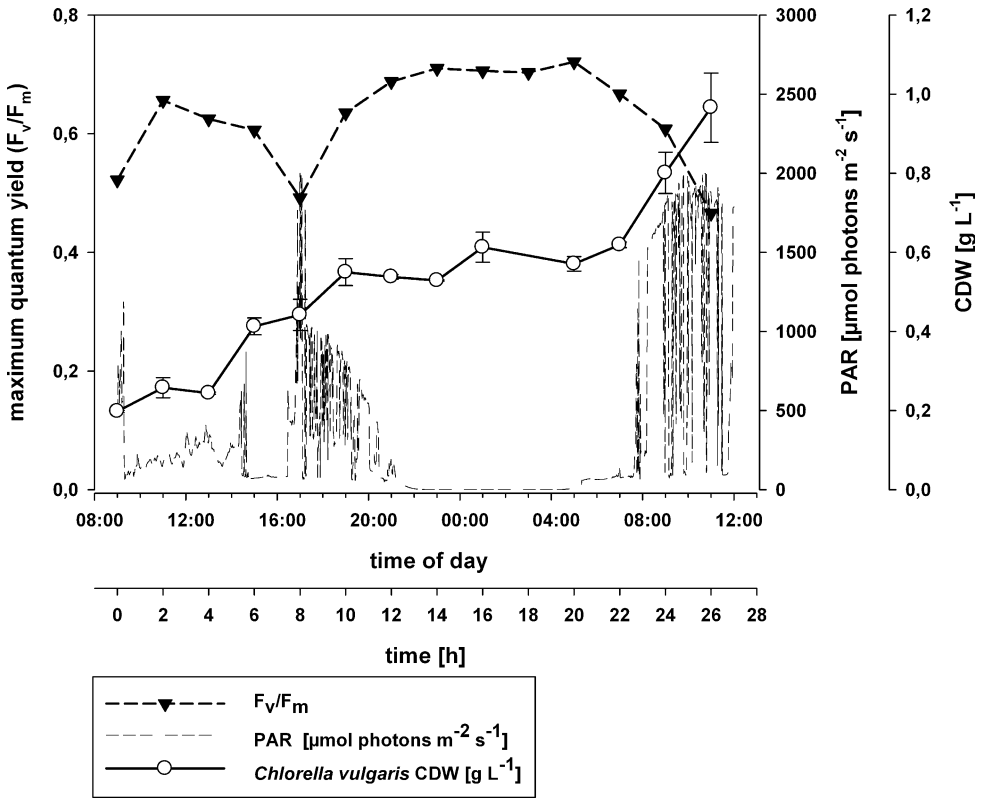

Fig. 4 Changes in the photosynthetic activity (F_v/F_m), cell density (CDW) and PAR during 27 h of outdoor cultivation of *C. vulgaris* in PBRs (OLP: 15 mm; 10 m^2 PBR surface) mounted on a solar tracker and exposed always perpendicular to the sun (standard mode)

Table 2 Daily amount of irradiance on a horizontal plane in comparison to photobioreactors mounted on a solar tracker and exposed at a constant perpendicular orientation to the sun (standard mode) or in a 180° angle to the sun (offset mode) during a clear summer day in Hamburg (Germany)

	Horizontal solar irradiance	Standard mode: maximum light	Offset mode: 90° minimum light
Mean PAR (µmol photons m^{-2} s^{-1})	636	923	222
Photons per day (mol m^{-2})	55	79	19
Percent (%)	100	145	35

decrease of F_v/F_m up to 25 % at high irradiance has indicated an inevitable heat dissipation of photosynthetic efficient energy which results in a loss of photosynthetic reduction equivalents for biomass production. The presented results were in accordance with those demonstrated by other authors [28–30]. Furthermore, it has been shown that below optimal biomass concentrations, artificial shading can increase the productivity of a *Spirulina* culture [14]. Therefore, the solar track technology was used to reduce the irradiance to 35 % of the horizontal irradiance (Fig. 3; Table 2). Below a microalgal concentration of about 1.1 g CDW L^{-1} (e.g. during start of the cultivation process), the cells were prone to light stress (Figs. 4, 5a). Thus, it is recommended that the PBRs should be operated in the offset mode to allow photoadaptation of the cells to the outdoor light regime. This set-up reduced the degree of photoinhibition because light stress was reduced by turning the reactors out of direct sun (Fig. 3).

Another possibility to reduce the degree of photoinhibition is increased self-shading by higher biomass concentrations. Hence, an experiment was performed to measure the degree of photoinhibition of a *S. obliquus* culture with increasing cell densities ranging from 0.5 to 4.6 g CDW L^{-1} over 11 days (Fig. 5a), as a representative batch run. During the first day of cultivation, irradiances below 600 µmol photons m^{-2} s^{-1} caused a relatively high F_v/F_m of 0.72, despite a low cell density of 0.5 g L^{-1}. During the second day (cell density still remained below 1.1 g L^{-1}), high midday PAR of up to 2,000 µmol photons m^{-2} s^{-1} caused strong photoinhibition with minimum F_v/F_m of 0.48, representing a degree of photoinhibtion of 40 %. Subsequently, F_v/F_m remained high between 0.74 and 0.80 over 9 days even during phases of high PAR up to 2,000 µmol photons m^{-2} s^{-1}, due to increased self-shading when biomass concentrations were above 1.1 g CDW L^{-1} (Fig. 5a).

On the first 3 days of cultivation, at low cell densities, the biomass yield was relatively low, around 0.4. The theoretical maximum biomass yield is 1.8 g CDW mol

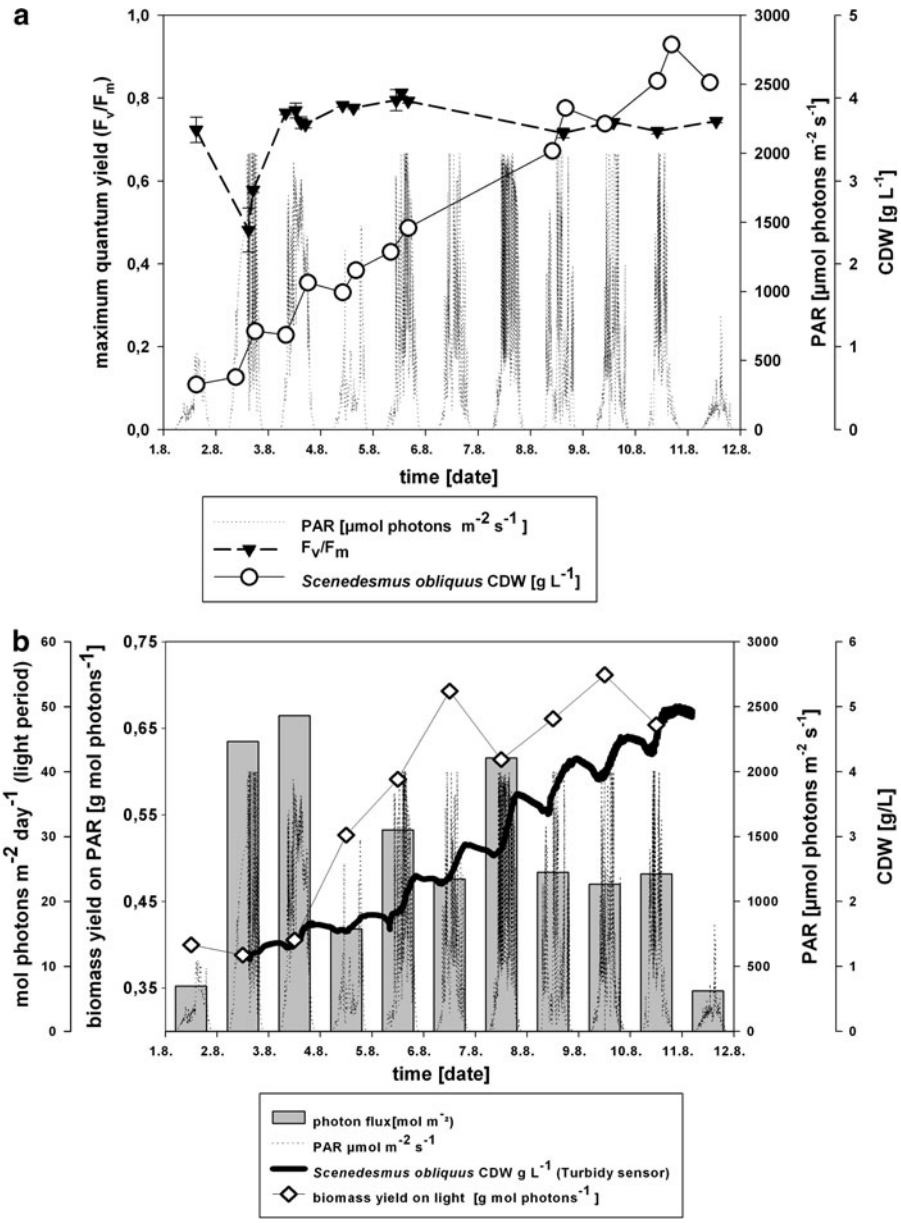

Fig. 5 Typical outdoor cultivation of *S. obliquus* with increasing biomass concentration in PBRs (OLP: 22 mm; 4 m^2 PBR surface) mounted on a solar tracker and exposed always perpendicular to the sun (standard mode). **a** Changes in the photosynthetic activity (F_v/F_m) and cell density (CDW). **b** Biomass yield for the different PAR doses per day (light period)

photons^{-1} for the growth on ammonia as nitrogen source [31]. Cuaresma et al. [31] demonstrated a high maximum biomass yield of *Chlorella sorokiniana* of 1.0 at the continuous irradiance of 2,100 µmol photons m^{-2} s^{-1} in a laboratory study. Of course, the PAR doses per day influenced the biomass yield even at higher cell densities; thus, in general, the biomass yield was higher at lower PAR doses per day. With rising biomass concentration, the biomass yield of *S. obliquus* increased to about 0.7 g mol photons^{-1} under outdoor conditions (Fig. 5b).

Short-term measurements of $\Delta F/F'_m$ were conducted in situ on the surface of the PBR to further elucidate the dependency of photoinhibition between irradiance and cell density (Fig. 6). The $\Delta F/F'_m$ of PS (II) of a light acclimated *Scenedesmus* culture increased at low and high PARs with rising biomass concentrations. In low density cultures with 0.5 g CDW L^{-1} and at a mean irradiance of 429 µmol photons m^{-2} s^{-1} (using the offset mode of solar tracker, Fig. 6), the $\Delta F/F'_m$ of the culture was 0.47. In contrast, at a high PAR of 1,560 µmol photons m^{-2} s^{-1} (standard mode of solar tracker), the $\Delta F/F'_m$ was low at 0.2. This showed that down regulation of the photosynthetic activity under high light was stronger than under low light. Under high light, the $\Delta F/F'_m$ values increased linearly with the increasing biomass concentration of 0.5–1.1 g L^{-1}. Reaching a biomass concentration of 2.7 g L^{-1}, the $\Delta F/F'_m$ started to approach its maximum of 0.68. Above this CDW, the $\Delta F/F'_m$ of the *Scenedesmus* culture was nearly constant even with further increases in the cell density. The maximum F_v/F_m value of a dark-

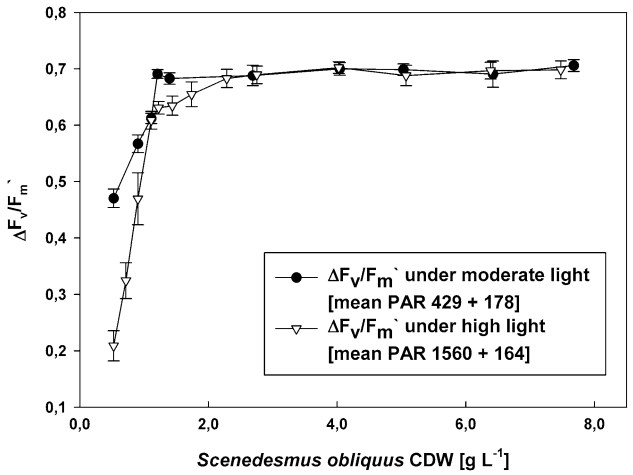

Fig. 6 Relationship between the $\Delta F/F'_m$ and the cell density of *S. obliquus* cultivated in a flat panel photobioreactor at moderate (offset mode) and high (standard mode) irradiances. Cell densities (7.5–0.5 g CDW L^{-1}) were adjusted by a stepwise dilution of the culture

adapted culture reached 0.80 at a CDW of 7.5 g L^{-1}. The results are, thus, in accordance with the long-term experiments of photoinhibition for both strains, *C. vulgaris* (Fig. 4) and *S. obliquus* (Fig. 5a). Consequently, the reduction of photoinhibition was successfully achieved by either increased self-shading by increasing biomass concentration or by the decreased irradiance via the offset mode of the solar tracker. Above a biomass concentration of 1.1 g L^{-1}, the PBRs were exposed perpendicular to the sun (standard mode or temperature mode) so that the algae culture of *S. obliquus* was able to utilize the high solar irradiances, with a tolerable low degree of photoinhibition (Fig. 6).

Enhanced irradiance by the solar tracker

The solar tracker enabled an increase of the irradiance of 45 % compared to the horizontal irradiance (Fig. 3; Table 2). This effect was even more pronounced during clear days in the winter season when the sun was at low altitude. The quantity of photons per day increased by about 55 % compared to the horizontal solar irradiance in winter. In summer, maximum PAR at the PBR surface was about 2,000 µmol photons m^{-2} s^{-1} during clear weather conditions. This high irradiance can be utilized by the microalgae due to adequate self-shading at biomass concentrations higher than about 2.3 g L^{-1} (Fig. 6). The high $\Delta F/F'_m$, e.g. 0.68 during PBR irradiance of around 1,500 µmol photons m^{-2} s^{-1}, indicates a high, non-inhibited photosynthetic efficiency and biomass productivity. Compared to the $\Delta F/F'_m$ determination, the biomass yield increased to a CDW of about 4 g L^{-1}, while the $\Delta F/F'_m$ already reached the maximum at a CDW of 2.7 g L^{-1} (Fig. 5b). This seems incongruent, but the in situ measurement represented only the activity of PS II and the biomass yield on light includes all metabolic processes within the generation of new biomass [32]. Thus, the optimum biomass concentration varied between a CDW of 2.3 and 4 g L^{-1} to maximize volumetric and areal productivity. A laboratory study demonstrated that higher irradiance resulted in higher optimum biomass concentrations for productivity, because light limitation was overcome [21]. This reveals that the increased irradiance results in higher biomass production of solar tracked systems compared with static systems. With respect to the OLP of 15–22 mm of the PBR used, the results correspond to the laboratory study of *Chlorella sorokiniana* in a turbulent mixed PBR (OLP: 12 mm; PAR: 1,500 µmol photons m^{-2} s^{-1}), where the optimum biomass concentration was 2.3 g L^{-1} [19]. In contrast to that, the optimum biomass concentration for a *Spirulina platensis* culture was between 12 and 18 g L^{-1} (OLP: 26 mm; PAR: 1,800 µmol photons m^{-2} s^{-1})[33]. A prerequisite is a short light/dark cycle for each cell, which is achieved by a high mixing rate of the culture medium [33]. With a suitable interplay among cell density, layer thickness and turbulence, the averaged cell irradiance decreases below the light saturation point and allows rapid light/dark cycles which possibly match the turnover of the photosynthetic apparatus [28, 34]. The usage of PBRs with a short OLP with high biomass concentrations facilitates further harvesting of the biomass [35]. The daily dose of absorbed photons is higher with the solar tracked system and leads to higher optimum biomass concentration and an additional increase in the volumetric productivity [18, 21, 31, 33]. For the cyanobacterium *Arthrospira platensis*, a theoretical simulation suggested an productivity increase from 35 to 40 t ha^{-1} $year^{-1}$ for a fixed horizontal PBRs to 55–60 t ha^{-1} $year^{-1}$ for solar tracked PBRs [36].

Temperature control by the solar tracker system

In the standard mode, the irradiation was increased to overcome light limitation, but simultaneously it also causes a temperature increase in the closed PBR system. At ambient temperatures similar to or above that in the PBR, heat cannot dissipate from the PBR. Therefore, especially in static outdoor PBRs with high surface to volume ratios, overheating of the culture medium occurs under high irradiances [12]. A large proportion of the solar irradiance consists of infrared radiation which is not utilized in photosynthesis and part of the visible range is converted to heat through protective non-photochemical heat dissipation [16, 32, 37]. In the standard mode, the solar track system increases the amount of impinging radiation, and consequently, an overheating of the culture medium is possible. On the one hand, the irradiance as one limiting photosynthetic factor should be totally absorbed by the PBR system. On the other hand, heat stress impairs algal growth [9].

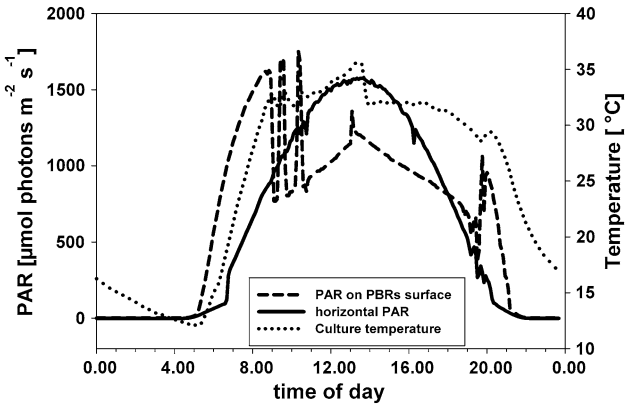

Fig. 7 Typical diel changes in temperature of a *S. obliquus* culture in PBRs during 6/3/2010. The irradiance was regulated by the solar tracker, using temperature mode, in order to maintain the temperature below the set value of 35 °C. For comparison, the photosynthetic active radiation (PAR) on a horizontal plane is given

Maximum light absorption caused a fast rise in temperature within 3 h from 12 to 35 °C, even at northern latitudes around 53° North (Fig. 7). The photosynthetic temperature optimum for *S. obliquus* is around 30 °C and for *C. vulgaris* 33 °C [38]. Therefore, the PBRs were operated in the temperature mode and the set value was set to 33 °C. Maximum values slightly above the optimum temperature of the cultivated microalgal strains of 35 °C were reached and the offset orientation was automatically enabled if the set value was exceeded, as a compromise between maximum irradiation and temperature optimum. This offset orientation reduced the temperature increase but unfortunately also the amount of absorbed irradiance.

An example for the horizontal and the irradiance on the PBRs as well as culture temperature are shown in Fig. 7, using the temperature mode. On one typical day (June 3 2010), the horizontal solar irradiance (PAR) was 52 mol photons m^{-2}, while the dose impinging on the reactor surface was only slightly higher with 55 mol photons m^{-2}. The advantage of the solar tracker, that a higher amount of PAR could be absorbed, was reduced by high medium temperatures during midday. However, in the morning and in the afternoon, more PAR reached the PBR compared to horizontal solar irradiance, when photosynthetic activity is highest during the natural diurnal photosynthetic cycle [39]. From 08:00 to 20:00 h, the temperature of the culture was close to the photosynthetic temperature optimum for both strains by enabling and disabling the offset and standard orientations. Hypothetically, the maximum daily dose could reach more than 80 mol photons m^{-2}, if the reactor is constantly perpendicularly exposed to the sun. Hence, only 68 % of the maximum daily dose had reached the surface of the PBR (Fig. 7) because temperature mode was enabled and cooling via a cold water aggregate was not applied. A commonly used method for controlling the temperature is water evaporation, where water is sprayed on the reactor surface [11]. The disadvantages of this method at a larger scale are both, the water consumption and production of salt crusts on the PBR surface. An alternative for cooling is a cold water aggregate in combination with an internal heat exchanger, which is in its test phase. Of course, the intensity of active cooling depended on the ambient temperatures and it was reduced during spring and autumn, when high PAR occurred with low ambient temperatures. In summer, a removal of thermal energy of about 76 W m^{-2} PBR surface (mean over 24 h) was necessary to obtain a mean culture temperature of 20.2 °C during 12 days of cultivation with a diel (24 h) average PAR of about 310 µmol photons m^{-2} s^{-1} and a mean air temperature of 19.8 °C (*Scenedesmus* cultivation Fig. 5a). In combination with heat exchangers, surplus energy absorbed by the PBR system might be used for heating of service water [40, 41]. If the surplus heat energy is used for further downstream processing, such as drying of biomass, a beneficial effect on an economic balance can be expected. This estimate is based on the assumption that heat demand for downstream processing, biomass productivity and heat availability are positively correlated to each other and show the same dynamics within different seasons. In this case, heat pumps could be an appropriate way to generate heat as a byproduct to biomass [42]. However, the cooling in the temperature mode needs no additional installation and has a low electric energy demand in comparison to heat pumps, but this goes along with reduced input of sun light. The beneficial effect of the combination of irradiance increase and regulation was demonstrated outdoors in the present study. Furthermore, waste heat for heating the microalgal culture in cold seasons at night seems to be a possible option to extend the operation time in cold unfavorable climates, where open pond system are no option. Flue gas was successfully used for outdoor algal mass production in this study and especially if flue gas and waste heat are supplied from the same source like rural biogas plant via combined block heat and power stations, synergetic effects are expected [22]. However, such a system needs a more sophisticated infrastructure and fast light/dark cycles must be applied, which increases the cost of construction and maintenance. Thus, economical production of bioenergy by this system is quiet unrealistic in the near future [42]. But higher valuable products, especially feed for aquaculture might be one possible way for local bioresource production.

Conclusions

With solar tracked PBRs, the microalgal cultures are higher irradiated (up to +45 %) than in a static system, which

leads to a higher optimum biomass concentration and volumetric productivity. Moreover, the culture temperature was regulated close to the species-specific optimum by the interplay of irradiance and ambient cooling. Due to the controlled light supply in the offset mode, it is also possible to provide low irradiance at low cell densities at the initial cultivation phase after inoculation (e.g. a small volume of preculture). This eliminates the necessity for additional shading or stepwise inoculation, as compared with other outdoor systems. In addition, this system may be useful to produce pre-cultures for large-scale production plants and may lead to shorter set-up times, e.g. for a change of the microalgae species with season or new strains for new products. If the higher costs of construction are compensated by higher biomass production and better performance of the culturing process, it is worth to be tested on a larger scale for specific bioproducts.

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Chapter 5 Key parameters for outdoor biomass production of Scenedesmus obliquus in solar tracked photobioreactors during different seasons

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The author of this dissertation conducted all preliminary and final experiments. The data were analyzed by multivariate statistics (Principal component analysis) to determine the interacting, multivariate dependencies within the outdoor production of microalgae and the manuscript were finished for publication.

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Key parameters for outdoor biomass production of *Scenedesmus obliquus* in solar tracked photobioreactors during different seasons

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Keywords: flue gas; outdoor microalgal biomass production; photosynthetic efficiency; *Scenedesmus obliquus*; solar tracked flat panel photobioreactors; temperature control

Abstract

The biomass productivity of *Scenedesmus obliquus* was investigated outdoors during all seasons in solar tracked flat panel photobioreactors (PBR) to evaluate key parameters for process optimization. CO_2 was supplied by flue gas from an attached combined block heat and power plant. Waste heat from the power plant was used to heat the culture during winter. The parameters pH, CO_2 and inorganic salt concentrations were automatically adjusted to non limiting levels. The optimum biomass concentration increased directly with the photosynthetic active radiation (PAR) from 3 g cell dry weight (CDW) L^{-1} to 5 g CDW L^{-1} for low PAR of 10 mol photons m^{-2} d^{-1} and high PAR 40 – 60 mol photons m^{-2} d^{-1}, respectively. The annual average biomass yield (photosynthetic efficiency) was 0.4 ±0.5 g CDW mol photons^{-1}. However, biomass yields of 1.5 g CDW mol photons^{-1} close to the theoretical maximum were obtained at low PAR. The productivity (including the night biomass losses) ranged during all seasons from -5 g CDW m^{-2} d^{-1} up to 30 g CDW m^{-2} d^{-1} with a mean productivity of 9 ±7 g CDW m^{-2} d^{-1}. Low night temperatures of the culture medium and elevated day temperatures to the species specific optimum increased the productivity. Thus, continuous regulation of the biomass concentration and the culture temperature with regard to the fluctuating weather conditions is essential for process optimization of outdoor microalgal production systems in temperate climates.

Highlights

- **Outdoor biomass productivity of microalgae in northern temperate climates**
- **CO_2 and waste heat usage supplied from a combined heat and power plant**
- **Application of solar tracked photobioreactors to increase light supply**
- **Evaluation of key parameters for process optimization**

1. Introduction

Microalgal biomass is discussed to supply sustainably the increasing demand of food, feed and biofuels cause of higher areal productivity and fertilizer uptake efficiency compared to other field crops [1, 2]. Additionally, microalgae production would not compete with cropland. Different production systems like open raceway ponds and closed photobioreactors (PBR) (horizontal-, vertical tube systems and flat plate PBR) were developed with system specific advantages and there is still an ongoing discussion which system is the most promising for specific bioproducts [3].

At photoautotrophic growth conditions, inorganic CO_2 is used as the only carbon source. Flue gas containing around 10 % CO_2 (v/v) and additionally traces of NO_x and SO_2 are supplied as an inexpensive C, N or S-sources in microalgal cultures [4-8]. Outdoor productivities fluctuate as they are influenced by the composition of the culture medium, the specific cultivation system and the light and temperature regime in different seasons and geographical locations. The cultivation system is characterized by the irradiance, optical light path (cross section), the mixing rate and process modes (batch, turbidostad etc.)[9]. Within the multifactorial systems identification of the dependencies between these parameters is prerequisite to optimize the productivity. However, the control of all culture conditions is possible only during indoor cultivation. Outdoors a control of culture temperature is energetically and economically demanding. For the development of a specific production system reliable data about the maximum biomass production capacity are essential. The maximum productivity of a specific cultivation system can by predicted once the solar energy to biomass energy conversion efficiency is known for different culturing conditions [10].

The theoretical maximum for photosynthetic efficiency (PE) is between 8-10 % of the light energy which correspond to productivity yield of 75±5 g CDW m^{-2} d^{-1} for the average irradiance in the US [11]. Another possibility to determine the photosynthetic efficiency is the biomass yield on light energy ($Y_{x,E}$)[12]. This specific $Y_{x,E}$ depends on the microalgae strain, the cultivation system and conditions like mixing rate, mass transfer, temperature regime and the nitrogen source. Once the $Y_{x,E}$ is known, multiplication with the photon flux density (PAR) gives the specific productivity [13], which is of main interest for the biotechnology community.

Simulation or modeling of the productivities for different location was done for a variety of strains and culture techniques [13-16]. However, in a multifactorial outdoor system the prediction of year-round productivities

based on laboratory data or short term experiments often overestimates the potential of different applications of microalgae biotechnology especial for the energy purposes [17]. Hence, robust, reproducible field data at least of pilot scale and long term measurements are prerequisite for the up scaling process of industrial scale production systems.

The specific aims of this study were to evaluate the key parameters which are limiting the outdoor biomass production of microalgae in northern temperate climates in the presented PBR system. The study included the use of waste heat and flue gas from a combined heat and power plant to enhance the biomass production. Thereby, flue gas was used as C-source for autotrophic growth and waste heat was supplied to overcome temperature limitation during winter. Experiments were done with *S. obliquus* which is known of high tolerance for flue gas, high growth rates and a cell composition of biotechnological interest [18, 19]. Solar tracked photobioreactors were used for both to increase the light supply and to manage culture temperature reduction by ambient cooling. The culture conditions inside the solar tracked PBRs were thus maintained constant within given limits and their effects on biomass productivity of *Scenedemus obliquus* were determined by multivariate statistics (Principal Component Analysis) using data obtained in outdoor experiments lasting from June 2010 to May 2012.

2. Materials and Methods

2.1 Pilot plant and photobioreactors

Flat panel PBRs mounted on a solar tracking system and located in Hamburg, Northern Germany (53°28'12"N; 10°10'40"E) were used for the outdoor experiments described herein (Fig. 1). Culture temperature was controlled by two independent techniques. Cooling was provided by a water cooler via a heat exchanger or by turning the PBR on the solar tracker out of the direct sunlight exposition in order to obtain a minimum solar energy absorbance ("temperature mode")[8]. Waste heat from a combined heat and power plant was used to raise the temperature above ambient temperature during winter. The combination of heating and cooling limited the fluctuation of culture temperature to between 5°C during night and/or low ambient temperatures during winter and 35°C during summer at high light intensities and high ambient temperatures.

Fig. 1 Outdoor system used in present study equipped with solar tracked PBRs and a temperature control by cooling and heating. Flue gas from a power plant was used as the only CO_2 source for the microalgae production.

Up to seven PBR panels were mounted on one solar tracker, each covering 2 m² and containing 30 - 45 L of microalgal suspensions with an optical light path (OLP) of 15 - 22 mm. The culture medium in the PBR circulated in an external tube system which included an analytical flow section equipped with probes for continuous measurements of pH, NH_4^+, NO_3^-, O_2 and turbidity. The external circuit also contained a retention tank to compensate variations in water volume which was used for feeding nutrients and to adjust

temperature. This circulation system increased the volume of the culture medium by 30 - 35 L and thus the total volume per solar tracker was 240 L maximum and the surface [m^2] to volume [m^3] ratio of the PBRs was 66 m^{-1}. Compressed air was periodically injected at the bottom of each PBR through valves at a frequency of 0.25 s^{-1} equivalent to a mean air flow of 0.4 L L^{-1} culture medium min^{-1} for mixing. In addition, flue gas was injected through the same valves at quantities necessary to maintain the pH value in the culture medium at below 7.5. The flue gas was obtained from a combined block heat and power station by burning natural gas and contained CO_2 at a concentration of around 8-10 % (v/v).

2.2 Microalgae strains, culture condition

The microalga *Scenedesmus obliquus (Chlorophyta)* used in present study was obtained from a previous microalgae screening for flue gas tolerating microalgae at the pilot plant and belongs to the SVCK algal collection at the University of Hamburg , No. U169.

For pre-culture production, the strain was grown in 10 L Schott glass flasks filled with 8 L water to which 2 g L^{-1} "Flory Basic Fertilizer 1" (Euflor, Germany) and 3.22 g L^{-1} KNO_3 were added. After 10 days of laboratory cultivation at 20°C, PAR of 100 µmol photons m^{-2} s^{-1}, and aeration with CO_2-enriched air (4 % v/v), the inoculum was added to the outdoor system to obtain a cell dry weight (CDW) of 0.1 g L^{-1}. Prior to inoculation the PBR device was cleaned with a 3-10% H_2O_2 solution for 48h and rinsed twice with tap water. The fresh outdoor culture was maintained at PAR ≤ 1000 µmol photons m^{-2} s^{-1} by rotating the PBR out of direct sunlight (offset mode) until the CDW increased to above 1 g L^{-1}.

The pH value was maintained between 6.5 and 7.5 by a combination of flue gas injections and titration of hydroxide. The upper pH value of 7.5 was established by varying the flue gas injection. A decrease of the pH below 6.5 was prevented by automatic titration with 5 N NaOH.

The oxygen concentration in the medium ranged between 4 - 18 mg L^{-1}. Nutrient concentrations were controlled by using the nitrate concentration as threshold. If nitrate decreased below a concentration of 100 mg NO_3^--N, the inorganic feeding media containing 60 g L^{-1} (Flory Basic Fertilizer 1) and 58.4 g L^{-1} NH_4NO_3 was automatically added to the culture. All experiments started with an axenic inoculum and were operated non-aseptically during further outdoor cultivation.

2.3 Determination of biomass formation and loss during the night

Biomass production was determined gravimetrically from the increase in the cell dry weight (CDW). 50 mL of culture medium was transferred into tubes and centrifuged 30 min at 6000 x g. The supernatant was discharged and the pellet was dried at 85°C for 48 h. Low biomass concentrations were determined by filtration of a 10 ml culture sample on pre-dried glass fibre filter GF6 (Whatman, Germany,) washed with 10 ml of distilled water. The CDW in g L^{-1} was calculated by comparison of the difference in weight of the pre-dried tube/filter and the tube/filter including the microalgae. No significant differences were found between the two methods.

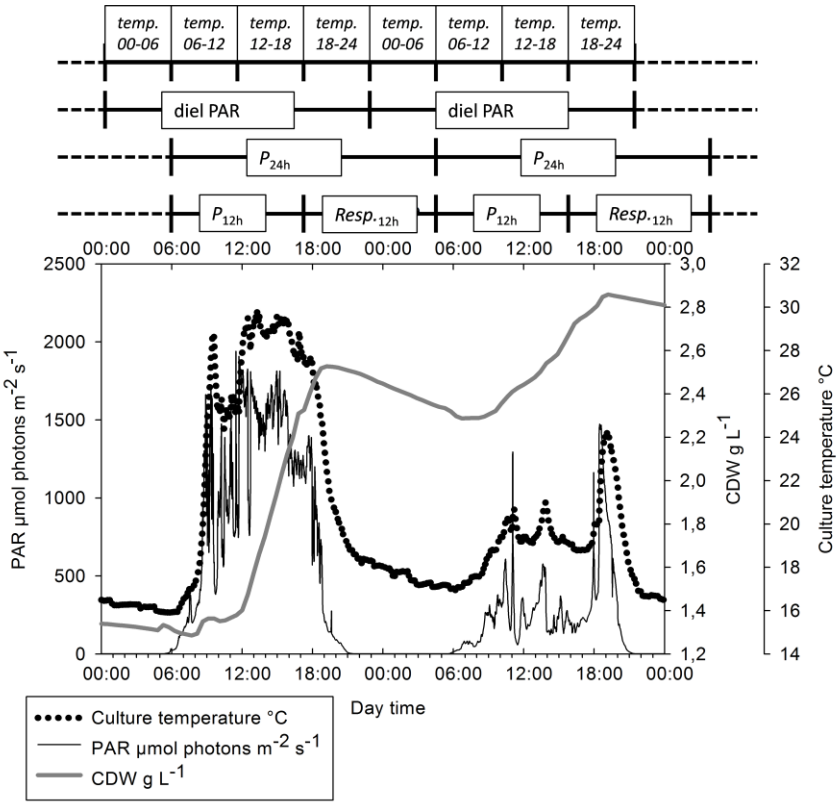

Fig. 2 Example for daily variations of photosynthetic active radiation (PAR), culture temperature and biomass concentration during outdoor cultivation to show the time intervals used presently to determine the effect of different culture conditions on productivity.

The productivity was calculated from the increase in CDW during a given time interval. The areal productivity was calculated from the volumetric productivity multiplied by the total culture volume (PBR volume + tube

volume) and divided by the area of PBR surface (A_{PBR}). The culture samples were taken every morning around sunrise as duplicates and the mean value was used, thus the presented productivities for 24h (P_{24h}) included all biomass losses like algal night respiration, bacterial activities and biomass losses by attachment or sedimentation in the tube system ($P_{24h} = P_{12h} - Resp._{12h}$). For determination of the biomass production during the light period (P_{12h}) the difference in CDW between the "sunrise" und the "sunset" sample was taken. The biomass losses ($Resp._{12h}$) were assessed by the difference in CDW between "sunset" und "sunrise" samples of the following day, respectively (Fig. 2). Cause of the different night lengths during different season of the year the measured values were interpolated to a standard night length of 12 hours for better comparison of the results. The corresponding CDW to a certain P_{24h} are the mean value of sunset and sunrise CDW concentration.

2.4 Light and photosynthetic efficiency

The irradiance of wavelengths between 400 and 700 nm (photosynthetic active radiation) was measured on the surface of the solar tracked PBR by a LI-COR probe (USA). Around 80% of the PAR transmitted through the PBR material. The PAR impinging on a horizontal plane (horizontal PAR) was used as a reference to the two operation modes of the solar tracking system.

Biomass yield ($Y_{x,E}$) on light energy (PAR) is given by equation (Eq. 1) as an indicator for the photosynthetic efficiency [12]. The areal productivity (P_{24h}) was determined for the time period (t) and the area of irradiated PBR surface [m^2].

$$Y_{X,E} = \frac{P_{area}\ [\text{g m}^{-2}\ \text{t}^{-1}]}{PAR\ photons\ [\text{mol m}^{-2}\ \text{t}^{-1}]} \qquad (1)$$

P_{24h} and $Y_{x,E}$ graphs were carry out by SigmaPlot 11.0 on a database of n=124 (days) and a running average of 0.6 and 0.4 sample proportion, respectively. P_{24h} for the absence of microalgae (CDW = 0 g L^{-1}) were predicted to be zero (n=10).

2.5 Principal component analysis (PCA)

The multifactorial dependencies of biomass productivity were analysed using a Principal Component Analysis (PCA). PCA is a dimension reduction technique that allows to reduce a large number of variables to few main components showing trends in the multivariate data [20]. Mean values determined from 114 measurements

obtained for each of the 17 variables were used for PCA. The variables included CDW, PAR_{24h}, P_{24h}, Y_{24h}, air temperature 00-24h ($T_{air00-24}$), culture temperature 00-06h (T_{00-06}), culture temperature 06-12h (T_{06-12}), culture temperature 12-18h (T_{12-18}), culture temperature 18-24h (T_{18-24}), culture temperature 06-18h (T_{06-18}), culture temperature 00-24h (T_{00-24}), pH value 00-06h and 18-24h ($pH_{00-06,\ 18-24}$), pH value 06-18h (pH_{06-18}), O_2 concentration mg L^{-1} 00-06h and 18-24h ($O_{2\ 00-06,\ 18-24}$), O_2 concentration mg L^{-1} 06-18h ($O_{2\ 06-18}$), NO_3^--N concentration mg L^{-1} 00-06h and 18-24h ($NO_{3\ 00-06,\ 18-24}^-$), NO_3^--N concentration mg L^{-1} 06-18h ($NO_{3\ 06-18}^-$). Since variables are of different units, the PCA was carried out using a correlation matrix instead a covariance matrix. In order to identify a subset of relevant main components, the Eigenvalues of the components below a random model (Broken Stick) were defined as not significant [21]. The analysis was carried out using the software STATISTICA version 2.16 and PAST [22].

3. Results

3.1 Light and temperature as the most important factor for the biomass productivity

The biomass productivity of the microalgae *S. obliquus* was studied in different experiment phases during two years (June 2010 – Mai 2012). With changing weather conditions over the year, the adsorbed PAR_{24h} varied from ≤ 1 to 62 mol photons m^{-2} d^{-1} with mean values of PAR of 37.5 mol photons m^{-2} d^{-1} during summer (May-July) and 3.8 mol photons m^{-2} d^{-1} in winter (Nov.-Jan.), respectively. The mean ambient air temperature ($T_{air00-24}$) during cultivation ranged from 4.6°C to 16.8°C and the mean temperature of the culture medium from 15.3° to 20.7°C, for winter and summer. As an effect of active heating and heating by solar radiation the culture was in average around 4°C warmer than the ambient air temperature. During the experiments the areal productivity also ranged from biomass losses of -5 g CDW m^{-2} d^{-1} up to 30 g CDW m^{-2} d^{-1} with a mean productivity of 9.1 g CDW m^{-2} d^{-1}. The results of the correlation matrix between the productivity and the main influencing factors defined in section 2.3 is given in addition to the mean and standard deviation in table 1 (Supplement 1 provides the complete dataset of n=114 days of determined P_{24h}). Culture temperatures averaged over different periods during the daytime showed a significant positive correlation (P_{24h} vs. T_{06-12} and T_{12-18}) (Tab. 1). In contrast a negative correlation was found between productivity (P_{24h}) and night culture temperature (T_{00-06}) (Tab. 1). A significant increase in the productivity with increasing PAR_{24h} indicated light limitation for most of the temperature conditions.

Tab. 1 Cross correlation between the variables effecting biomass productivity including means and standard deviations. **Underlined marked** correlations are significant for $p < 0.05$.

	P_{24h}	PAR_{24h}	CDW	Y_{24h}	$T_{air00-24}$	T_{00-06}	T_{06-12}	T_{12-18}	T_{18-24}	T_{06-18}	T_{00-24}	$pH_{00-06, 18-24}$	$O_{2\ 00-06, 18-24}$	$NO_3^-{}_{00-06, 18-24}$	pH_{06-18}	$O_{2\ 06-18}$	$NO_3^-{}_{06-18}$
P_{24h}	1																
PAR_{24h}	**0,56**	1															
CDW	**0,21**	0,38	1														
Y_{24h}	**0,33**	-0,18	-0,11	1													
$T_{air00-24}$	0,16	0,17	0,21	-0,2	1												

T_{00-06}	**-0,23**	-0,37	-0,16	-0,08	0,56	1											
T_{06-12}	**0,2**	0,32	0,35	-0,18	0,62	0,54	1										
T_{12-18}	**0,29**	0,53	0,37	-0,22	0,62	0,14	0,7	1									
T_{18-24}	-0,02	0,04	0,20	-0,18	0,71	0,62	0,64	0,66	1								
T_{06-18}	**0,27**	0,47	0,39	-0,22	0,67	0,34	0,9	0,94	0,7	1							
T_{00-24}	0,10	0,19	0,25	-0,21	0,75	0,65	0,87	0,79	0,86	0,89	1						
$pH_{00-06,\ 18-24}$	**-0,22**	-0,28	-0,2	0,14	0,09	0,15	-0,11	-0,11	0	-0,11	-0,02	1					
$O_{2\ 00-06,\ 18-24}$	**-0,3**	-0,36	-0,6	0,19	-0,31	0,12	-0,25	-0,32	-0,09	-0,31	-0,16	0,2	1				
$NO_3^-{}_{00-06,\ 18-24}$	0,03	-0,16	-0,07	0,13	-0,14	0,01	-0,02	-0,08	-0,09	-0,05	-0,05	0,07	-0,05	1			
pH_{06-18}	-0,16	-0,08	-0,08	0,05	0,12	0,02	0,02	0,08	0,02	0,06	0,06	0,85	0,07	0,1	1		
$O_{2\ 06-18}$	-0,05	0,05	-0,51	0,11	-0,33	-0,2	-0,26	-0,19	-0,27	-0,24	-0,26	0,19	0,71	0,01	0,19	1	
$NO_3^-{}_{06-18}$	0,08	-0,11	-0,06	0,12	-0,23	-0,03	0	-0,09	-0,14	-0,05	-0,08	-0,07	-0,05	0,87	-0,03	0,05	1
Parameter mean	**9,14**	**25,79**	**2,4**	**0,41**	**15,11**	**14,35**	**18,92**	**24,28**	**18,56**	**21,6**	**19,03**	**7,11**	**6,95**	**102,88**	**7,24**	**9,28**	**100,15**
SD	**6,97**	**14,6**	**1,86**	**0,53**	**5,9**	**4,15**	**4,21**	**5,16**	**4,16**	**4,32**	**3,59**	**0,3**	**1,97**	**66,79**	**0,33**	**2,65**	**60,12**

In order to reduce the number of variables and to identify the most important ones for biomass productivity a PCA was carried out. Based on a broken stick model, a subset of 2 principal components was chosen. These explained 49.7 % of the variance in the dataset (Supplement 2 provied all principal components and the corresponding Eigenvalues). The first principal component was mostly influenced by the temperature variables (Fig. 3). The second principal component represented the variation of CDW, PAR_{24h}, P_{24h}, the pH values and the oxygen concentration in the culture medium ($pH_{\ 00-06,\ 18-24}$, $pH_{\ 06-18}$, $O_{2\ 00-06,\ 18-24}$, $O_{2\ 06-18}$) (Fig. 3). Inverse correlations were found for P_{24h} versus pH-values and O_2 concentration. Thus, a low O_2 concentration in the medium was connected to increased photosynthetic activity. The injection of flue gas was regulated by keeping the pH value constant at 7.5 and thus CO_2 limitation was excluded at any production rate. The nitrate concentrations only contributed marginal to the variation of the first and second component as indicated by their relatively short vectors (Fig. 3). Nitrate concentration was kept always close to 100 mg NO_3^--N L^{-1} by constant feeding, hence, this result was expected.

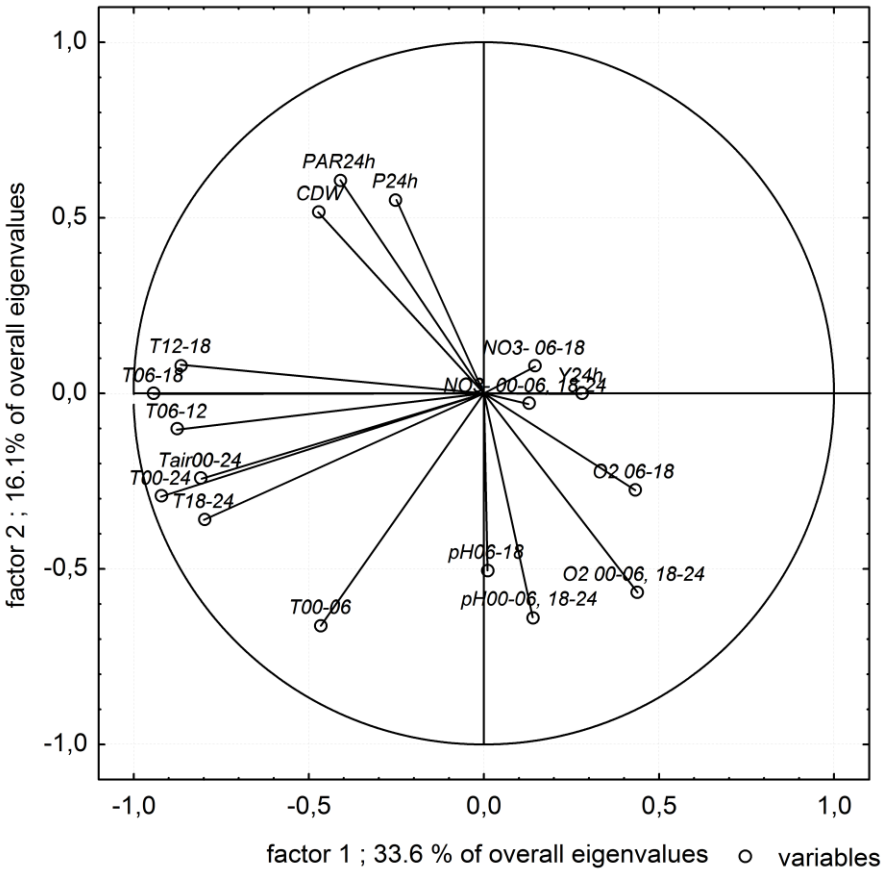

Fig. 3 Vectorial projection of the Principal Component Analysis showing all variables under study. Vectors showing the same direction revealed high colinearity. Vectors having opposite directions show negative correlations. The lengths of the vectors indicate their importance in relation to the other vectors.

3.2 Outdoor productivity

The cross matrix analysis (Table 1) showed significant positive correlations between P_{24h} and CDW concentrations and the PAR_{24h}. In order to further elucidate the dependency, the results from all experiments were plotted in a three dimensional graph (Fig. 4A). The optimum biomass concentration was around 3 g L^{-1} at low light conditions below PAR_{24h} of 10 mol photons m^{-2} d^{-1}. With increasing PAR_{24h} the optimum biomass concentration rose to about 5 g L^{-1}. Maximum average productivities were at maximum PAR_{24h} of 60 mol photons m^{-2} d^{-1}. At these high light conditions the average areal productivity was between 15 and 20 g m^{-2} d^{-1}. It was observed that the productivity drastically decreased once the culture was maintained above the optimum biomass concentration, especially at low light intensities below PAR_{24h} of 10 mol photons m^{-2}. At these light conditions biomass degradation during the night was nearly equal to its formation in the light

period. This was especially relevant during the winter cultivation from December to January were the productivity did not exceed 5 g DW m^{-2} d^{-1} and at some days biomass loss were measured. The biomass loss (*Resp.* $_{12h}$) in the night was in average 6 ±4% of the biomass concentration. Based on the same data the averaged biomass yield Y_{24h} is shown in figure 4B.

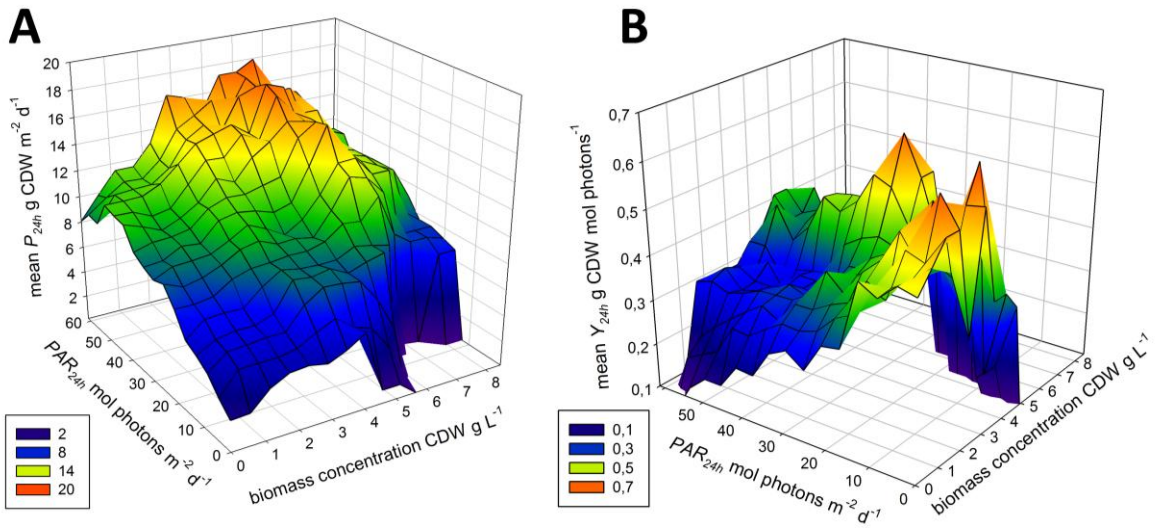

Fig. 4 Dependency of the averaged biomass productivity (A) and the averaged biomass yield (B) on the sum of photosynthetic active radiation reaching the photobioreactors during one day (PAR_{24h}) and the biomass concentration (CDW) in the culture medium. Color coding gives average values of the biomass productivity (A) and the averaged biomass yield (B).

3.3 Photosynthetic efficiency

The averaged Y_{24h} versus the CDW and PAR_{24h} reached the maximum of 0.6 below PAR_{24h} of 20 mol photons m^{-2} and a biomass concentration between 2 and 6 g L^{-1} CDW (Fig. 4B). With increasing PAR_{24h} the average Y_{24h} lowered to around 0.3 at maximum PAR_{24h} of 60 mol photons m^{-2} d^{-1}. Local minimum values were found either at high CDW and low PAR_{24h} or at low CDW and high PAR_{24h}.

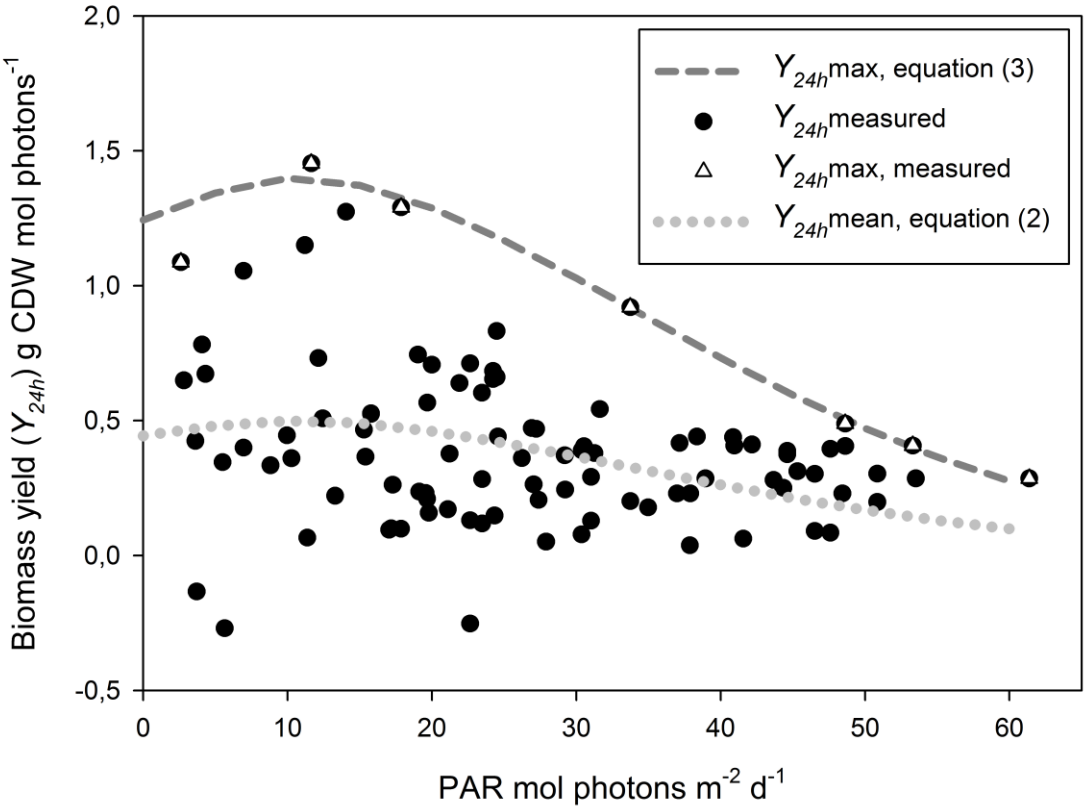

Fig. 5 Correlation between daily biomass yields (Y_{24h}) and photosynthetic active radiation (PAR_{24h}). Short dashed dark gray line is representing the maximum Y_{24h} values (Eq. 3) and the dotted line describes the mean Y_{24h} for a specific PAR_{24h} (Eq. 2).

The Y_{24h} generally decreased with increasing PAR (Fig. 5). At PAR_{24h} higher than 40 mol m^{-2} d^{-1} Y_{24h} were always below 0.6. While at PAR_{24h} below 20 mol m^{-2} d^{-1} Y_{24h} values up to 1.5 were found. The Y_{24h} showed its maximum of 1.5 g CDW mol photons^{-1} at PAR_{24h} of 12 mol m^{-2} d^{-1} and a biomass concentration of 0.8 g CDW L^{-1}. The mean Y_{24h} in dependency of the PAR_{24h} is given by the equation 2 calculated from the results shown in Fig 5. The maximum Y_{24h} values measured can be described with good approximation by the equation 3, (Fig. 5). Up to PAR_{24h} of 12 mol m^{-2} d^{-1} Y_{24h} were increasing followed by a linear decrease of Y_{24h}. Both equation (2) and (3) derived from a modified Gaussian approximation with four parameters.

$$Y_{24hmean} = 0.5 * exp^{-0.5} * \left(\frac{(PAR_{24h}-11)}{25} \right)^{1.75} \qquad (2)$$

$$Y_{24hmax} = 1.4 * exp^{-0.5} * \left(\frac{(PAR_{24h}\text{-}11)}{25} \right)^{1.75} \quad (3)$$

3.4 Modeling of biomass productivity for solar tracked photobioreactors

The availability of sunlight for the biomass production changes drastically during the year. Solar tracked PBRs were used to increase the irradiance per PBR surface and the overall irradiance was higher by 147% compared to horizontal irradiance (100%), prerequisite was the prevention of overheating of the culture temperature via a cold water aggregate (Fig 6. Aug 2011 until April 2012). A special operation mode called temperature mode was applied to keep the culture temperature below set value without the need of additional cooling. In the temperature mode the PBR were exposed to maximum irradiance as long as the culture was below 35°C as described in detail before [8]. Over the period from June 2010 to July 2011 the mean horizontal PAR was 19.1 and the PAR measured on the solar tracked photobioreactors was nearly equal with 19.3. However without additional cooling by a cold water aggregate the irradiance on solar tracked PBR was lower to 84% compared to the horizontal irradiance because ambient cooling was inefficient to compensate culture heat up (Fig. 6 April until July 2011).

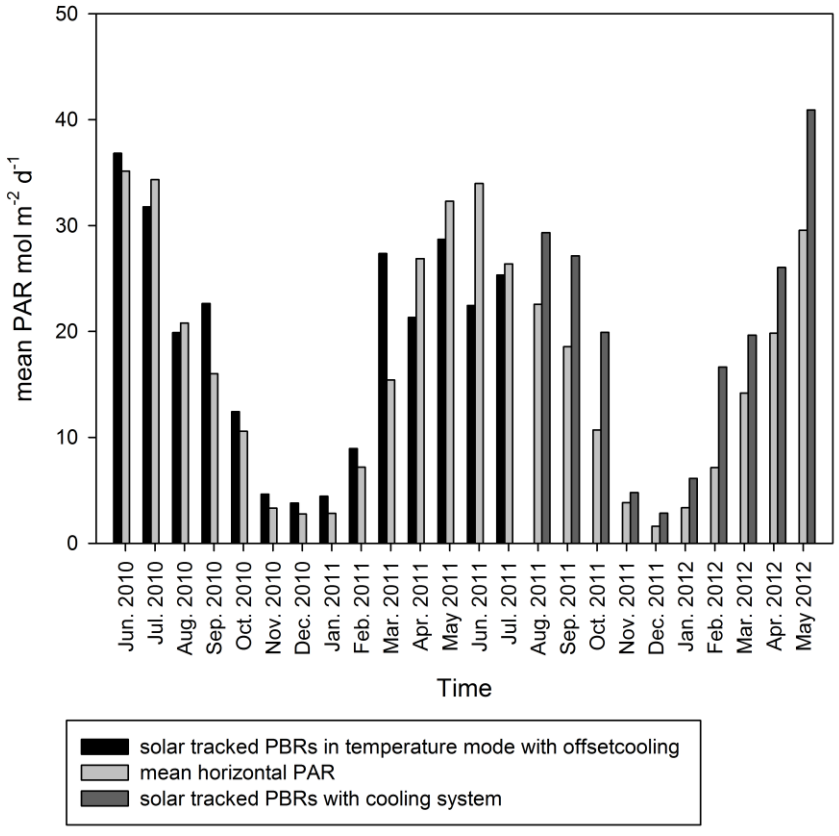

Fig. 6 Mean photosynthetic active radiation (PAR) which irradiated the solar tracked PBRs per month. The dark columns show the mean PAR_{24h} during operation in the temperature mode, were the photobioreactors are turned out of the sun (offset cooling) when the temperature set values was reached. The dark grey represent the mean PAR_{24h} for the standard mode (maximum PAR). Overheating of the culture was prevented using heat exchangers and a cooling system. Light gray (horizontal PAR) is given as a reference.

In this study two simple calculations were used for the modulation of the year round productivity base on the measured Y_{24h} values for different PAR_{24h}. First of all a non-optimized scenario is given by the equation (4) were the specific mean Y_{24h} is multiplied with the mean PAR_{24h} for each month. Secondly an optimistic scenario is presented for the maximum Y_{24h} which were determined for this specific cultivation system (Eq. (5))(Fig. 6).

$$P_{24hmean} = PAR_{24h} * 0.5 * exp^{-0.5} * \left(\frac{(PAR_{24h}-11)}{25} \right)^{1.75} \quad (4)$$

$$P_{24hmax} = PAR_{24h} * 1.4 * exp^{-0.5} * \left(\frac{(PAR_{24h}-11)}{25} \right)^{1.75} \quad (5)$$

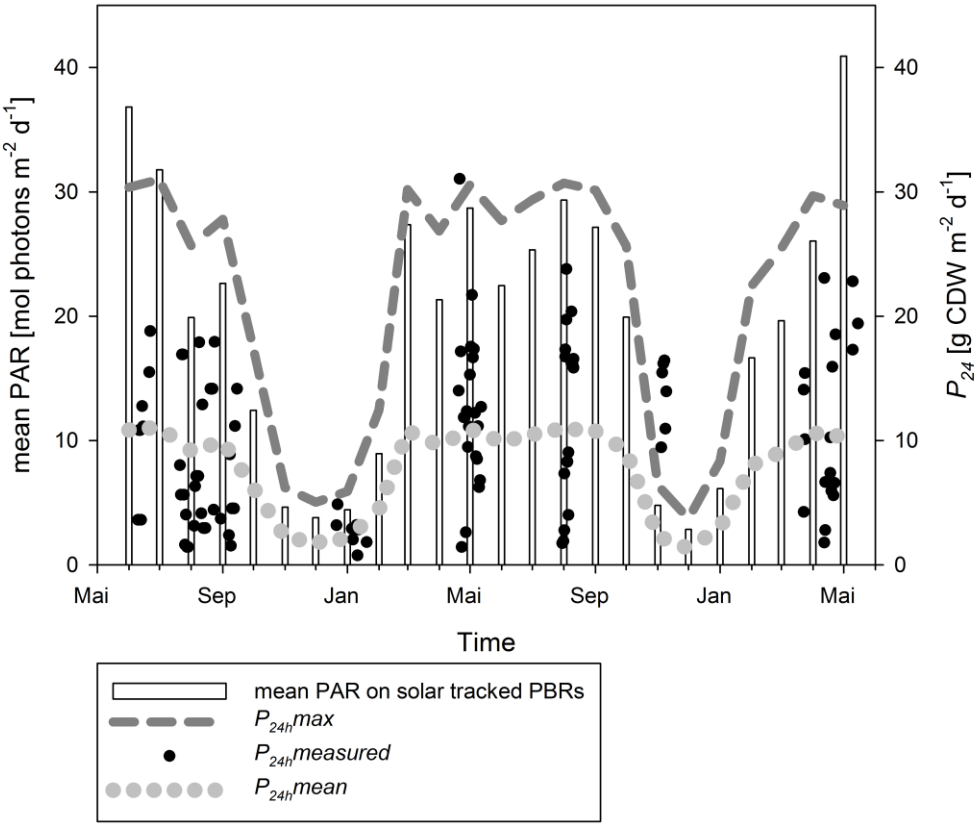

Fig. 7 The PAR_{24h} on solar tracked PBRs and the observed biomass productivity ($P_{24h}measured$) are shown. The $P_{24h}max$ and the $P_{24h}mean$ line are calculated productivities from the Eq. (4) and the Eq. (5) as the product of Eq. (2) and Eq. (3) multiplied with the irradiance (PAR_{24h}).

The predicted areal productivity (optimum scenario) for solar tracked PBRs is around 30 g CDW m^{-2} d^{-1} from March to September (Fig. 7). 78 % of annual biomass production would be generated between March and September due to different light availability distribution within the year. In winter (November to January) also for best-case production productivity cannot exceed 10 g CDW m^{-2} d^{-1}. The mean values are 21.5 and 7.7 for optimum scenario and non optimized scenario, respectively. Thus optimized culture conditions increased the productivity by almost a factor of three compared to the measured mean values.

4. Discussion

4.1 Key parameters for outdoor productivity

Of the different parameters presently under study light, CDW and temperature were identified as the parameters which mostly affected the productivity in outdoor cultivation. Dissolved nitrogen and CO_2 described in literature as key parameters for productivity in laboratory experiments had no effect because these were maintained in the present study by an automatic feeding device always at non limiting concentrations of ≥ 50 mg L^{-1}. Also S, P and trace elements are known to become key parameters if limited and to affect both productivity and biomass composition. Each algal species has a specific demand for these depending on the composition of their biomass which varies for nucleic acid between 3-5%, polysaccharides between 10-50% (mean 27%), lipids between 15-60% (mean 24%) and proteins between 20-60% (mean 48%) [23]. Because *Scenedesmus obliquus* was cultured under non limiting nutrient and trace element conditions these did not limit the biomass productivity. The pH was also not a limiting factor in the present study because of the strict control via flue gas addition which allowed maintaining the pH value always between 6.5 and 7.8. In contrast, a study about *Spirulina sp.* cultivation in open ponds suggested that the pH values was the most imported factor regarding the biomass productivity [24].

4.2 Effect of the availability of light on productivity

The solar radiation differs with the latitudes and the weather conditions [23, 25]. The annual mean of solar radiation lowers from 25 MJ m^{-2} d^{-1} at equatorial regions to around 10 MJ m^{-2} d^{-1} at latitudes of 50° – 60° [23]. In the present study the lower availability of light can be compensated by using solar tracked PBRs which resulted in a surplus of 147% of PAR based on PBR surface compared to the irradiance on the horizontal plane (Fig. 6). It was shown that with increasing daily doses of PAR (PAR_{24h}) the CDW in the culture medium has to be increased to obtain optimum productivities (Fig. 4). The productivity of microalgae cultures is the result of the photosynthetic rate of each single microalgae cell affected by the immediate irradiance. The photosynthetic electron turnover is known to occur in the order of nano- to microseconds. Also the adaptation by the light harvesting pigments can be at least one order of magnitude faster than a daily radiation cycle [26]. Thus, for photosynthesis the photon flux density in the time scale of the photosynthetic reactions (millisecond to minutes) is more important than the time scale of a day. The intense mixing caused that the microalgae are

exposed for very short periods to maximum PAR below the surface of the PBR. The irradiance regime of a single cell could not been determined due to the complex flow dynamics by airlift mixing. Furthermore, the same amounts of photons m^{-2} d^{-1} (PAR_{24h}) were detected during a short but clear winter day and during a long cloudy summer day. Hence, it is thus more appropriate to use PAR_{24} instead short term light intensities to calculate conversion efficiencies of light into biomass in order to determine the performance of an outdoor cultivation plant. Also the regulation of the CDW to obtain optimum productivity should be done with respect to PAR_{24} rather than to the actual PAR.

The control of the self shading effect by varying the biomass density (light attenuation properties) is one of the most imported factors regarding the photoinhibition in photobioreactors with a short OLP [8]. Previous study of Hartig et al. 1988 for *S. obliquus* are in agreement with the presented results that the areal biomass density is one of the key parameters to maximize the productivity [27]. Also for open race way ponds it was observed the that 20 % for medium and 60 % for low density cultures were “silenced” reaction centers at high irradiance during midday [9]. In contrast to traditional field crops the adjustment of the operated biomass concentration (OLP) is possible via culture dilution or partial harvesting. Both allow to adjust the light attenuation characteristics of the culture and to vary the frequency of light/dark cycles in well-mixed photobioreactors. The areal productivity with the OLP of 15 to 22 mm showed an optimum biomass concentration between 3 and 5 g CDW L^{-1} which was in the range reported for comparable outdoor systems were *Monodus subterraneus* was cultivated [28].

An operation of PBRs at the optimum biomass concentration and not at the maximum possible for a specific system relies on an energy efficient harvesting concept because more culture volume is needed to be processed. The dewatering step plays a major role of the downstream processing. Hence, dissolved air flotation and suspended air flotation are possible solutions and the combination with bioflocculation by photosynthesis will improve the energy balance of the operation concept [29-33]. Due to the accumulation of C- storage products, which increases the C/N ration during the day [34], the optimum harvesting time would be at the end of the light period (see Fig. 2). Thus the $Resp._{12h}$ is reduced because of the lower biomass concentration. This is an important factor for biotechnological approaches using microalgae instead of traditional biomass sources.

4.3 Biomass yield on PAR

The maximum $Y_{x,E}$ differs with the supplied N-source. For the growth on NH_4^+ 1.8 g CDW mol photons^{-1} are assumed [35] and for the growth on nitrate 1,57 g CDW mol photons^{-1} [36]. In present study using both NH_4^+ and NO_3^- for cultivating *S. obliquus* the Y_{24hmax} was close to the theoretical maximum of 1.5 g CDW mol photons^{-1} at PAR_{24h} of 12 mol photons m^{-2} d^{-1} and a biomass concentration of 0.8 g CDW L^{-1}. At higher PAR_{24h} the Y_{24hmax} lowered accordingly to the equation (3).

For indoor studies, maximum $Y_{x,E}$ values of 1.0 were determined for *Chlorella sorokiniana* at a constant PAR of 2100 µmol photons m^{-2} s^{-1} and at the optimum temperature of 37 °C (Cuaresma et al. 2009). Such a high irradiance equals a daily dose of 91 mol photons m^{-2} d^{-1} (light/dark cycle = 12h/12h) and this high corresponding $Y_{x,E}$ could not been reached outdoors with solar tracked PBRs at a latitude of 53°. Hence, the outdoor biomass production will always be below an indoor production with optimal light conditions.

The theoretical maximum PE is suggested to be 8-10% (energy conversion) or equivalent to 77 ±5 g CDW m^{-2} d^{-1} for the average irradiance of 35 mol photons m^{-2} d^{-1} [11]. However a review of published outdoor microalgal productivities for PBR systems found maximum productivities around 40 g CDW m^{-2} d^{-1} in averaged [23]. In the present study, the mean annual productivity was 9.1 g CDW m^{-2} d^{-1} and accounted for only 30 % of the maximum productivity of 31 g CDW m^{-2} d^{-1}. However, assuming the establishment of optimized culture conditions and solar tracking (Eq. (5)) an annual mean productivity of 22 g CDW m^{-2} d^{-1} was calculated which equals to 78 t CDW ha^{-1} of photoactive PBR surface year^{-1} at a latitude of 53°. This prediction based on optimum CDW and temperature course of the culture and no fertilizer limitation including CO_2. However productivity interpolation over more than three orders of magnitude in scale (from a few square meter to ha) is often not reliable [17] and interpolated production rates should be handled which caution.

4.4 Temperature

The waste heat supply enabled the continuous cultivation in northern latitudes where strong variations in daily mean air temperatures between -5°C and 27°C occurred during winter and summer. These variations were reduced in the outdoor cultivation by heating and cooling and temperature was always maintained in the culture medium at daily mean temperatures of 9°C - 25°C. A decrease of 10°C from the optimum temperature was found in laboratory to produce a decreased of $Y_{x,E}$ from 1.2 to 0.5 g CDW mol photons^{-1} for *Chlorella sorokiniana* [37]. In the present study the overall mean temperature of the culture was 19°C which is 11°C

below the optimum temperature of 30°C [38]. In accordance with the literature cited above, at these suboptimal culture temperatures a mean value of $Y_{x,E}$ of 0.41 g CDW mol photons^{-1} was determined.

Statistical analysis of the effect of the night temperature ($T_{00\text{-}06}$) revealed that it was negatively correlated to P_{24h} and positively correlated to elevated temperatures during the daylight (Tab. 1). This leads to the conclusion that lower night temperatures and elevated temperatures close to species optimum during sunlight maximizes the productivity. Similarly, a heating of the culture medium in the morning was found to improve the productivity [39]. Prerequisite are efficient heat exchangers and an economic energy source like waste heat from electric power plants and should be implemented only if the energy consumption for a year round temperature regulation is compensated by the biomass production.

Increased temperature during the night produce increased losses due to respiration. Because optimum productivity at higher irradiance demands for an increase in CDW, respiratory losses increase with these. In present study mean losses of 6 % were determined which are in accordance with former studies were the overall biomass loss during 12 h of darkness was 2- 10% [40]. With respect to optimizing culture conditions it can be concluded, that at higher CDW the temperature should be maintained during the night below 10°C and increased to optimum temperatures for photosynthesis during daylight.

4.4 Conclusion and outlook

CO_2 limitation was overcome by the presented CO_2 supply via flue gas and the integration of waste heat usage from a combined heat and power plant reduced the dependency of ambient temperatures. This enabled a year round production of microalgal biomass at unfavorable, fickle climatic conditions. The application of solar tracked photobioreactors demonstrated the increased light supply compared to static horizontal photobioreactors. 78 % of the annual biomass production is generated between March and September due to the sun light distribution within the year. The results revealed that the most important key parameters for process optimization are the biomass concentration and the culture temperature with regard to the fluctuating weather conditions. Optimization of the key parameters resulted in a threefold increase of the biomass productivity (mean productivity vs. optimum productivity, Fig. 7).

In future it is worth to test the integration of microalgae cultivation with traditional land based energy crop cultivation. Synergetic effect can be expected if the microalgae biomass production concept includes an

anaerobic biogas plant, where atmospheric CO_2 is fixed by energy crops [41]. CO_2 for the microalgae growth can be used either from row biogas or after the electrification of the biogas from the flue gas which is similar to the flue gas used in this study. Additionally waste heat is generally available if the biogas is electrified and the digestate can replace petro based N-fertilizer depending on the product quality generated by the microalgae.

Abbreviation

A_{PBR}	illuminated photobioreactor surface [m^2]
CDW	cell dry weight, [g]
NO_3^- 00-06, 18-24	mean NO_3^- concentration of the culture between 00:00 h and 06:00 h; 18:00 h – 24:00 h
NO_3^- 06-18	mean NO_3^- concentration of the culture between 06:00 h and 18:00 h
OBC	optimum biomass concentration [g L^{-1}]
OLP	optical light path [mm]
O_2 00-06, 18-24	mean O_2 concentration in the culture between 00:00 h and 06:00 h; 18:00 h – 24:00 h
O_2 06-18	mean O_2 concentration in the culture between 06:00 h and 18:00 h
PAR	photosynthetic active radiation (µmol photons m^{-2} s^{-1}, 400–700 nm); horizontal PAR (PAR absorbed by a horizontal plane)
PAR_{24h}	diel PAR mol m^{-2} d^{-1}
PBR	photobioreactor
P_{24h}	areal Productivity, formation of biomass (CDW) in 24h per illuminated photobioreactor surface
P_{12h}	areal Productivity, formation of biomass (CDW) in (main daylight time)

PE	Photosynthetic efficiency % (Energy content of the generated biomass/Energy of supplied light)
$pH_{00\text{-}06,\ 18\text{-}24}$	mean pH value of the culture between 00:00 h and 06:00 h; 18:00 h – 24:00 h
$pH_{06\text{-}18}$	mean ph value of the 06:00 h and 18:00 h
R_{12h}	Respiration (including all biomass losses) in 12 hours of darkness g L^{-1}
$T_{air00\text{-}24}$	mean air temperature °C between 00:00 h and 24:00 h
$T_{00\text{-}06}$	mean culture temperature °C between 00:00 h and 06:00 h
$T_{06\text{-}12}$	mean culture temperature °C between 06:00 h and 12:00 h
$T_{12\text{-}18}$	mean culture temperature °C between 12:00 h and 18:00 h
$T_{18\text{-}24}$	mean culture temperature °C between 18:00 h and 24:00 h
$T_{06\text{-}18}$	mean culture temperature °C between 06:00 h and 18:00 h
$T_{00\text{-}24}$	mean culture temperature °C between 00:00 h and 24:00 h
V	culture volume [L]
X_{CDW}	biomass concentration, [g L^{-1}]
$Y_{x,E}$	biomass yield (biomass formation on PAR photons) g CDW mol $photons^{-1}$
Y_{24h}	biomass yield over 24h (including biomass loss during the night)

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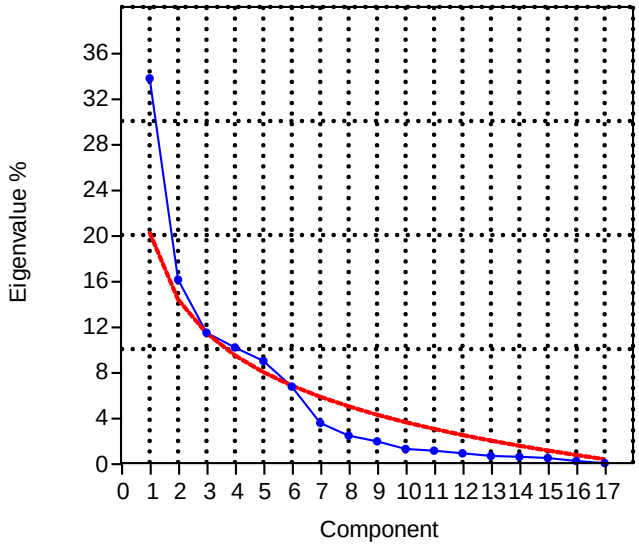
Supplementary table 1 Basic data set of mean values for 17 variables regarding and including the biomass productivity of *Scenedesmus obliquus*. *marked days (21.- 22.12.2010 and 06.01.2011) did not represent reliable irradiance (PAR values) due to snow cover on the light measuring sensor. Thus the incorrect biomass yield for these tree sample points were ignored.

Date	Production Unit	CDW g L^{-1}	PAR mol m^{-2} d^{-1}	Productivity g CDW m^{-2} d^{-1}	Yield g CDW mol photons^{-1}	Air temperature °C mean 00-24h	Culture temperature °C mean 00-06h	Culture temperature °C mean 06-12h	Culture temperature °C mean 12-18h	Culture temperature °C mean 18-24h	Culture temperature °C mean 06-18h	Culture temperature °C mean 00-24h	mean pH 00-06h, 18-24h	mean O2 mg L^{-1} 00-06h, 18-24h	mean NO_3^- mg L^{-1} 00-06,h 18-24h	mean pH 06h-18h	mean O2 mg L^{-1} 06h-18h	mean NO_3^- mg L^{-1} 06h-18h
10.06.2010	L1	0,5	21,1	3,6	0,2	20,7	18,0	19,5	24,2	19,9	21,8	20,4	7,2	8,0	54,4	7,3	10,1	55,7
11.06.2010	L1	0,7	24,6	10,8	0,4	20,1	17,8	20,7	27,2	19,2	24,0	21,2	7,2	8,3	52,0	7,3	10,2	51,5
12.06.2010	L1	1,2	29,2	10,8	0,4	15,5	15,5	20,7	20,3	17,6	20,5	18,5	7,3	9,6	54,6	7,5	12,2	52,3
13.06.2010	L1	1,8	24,3	3,6	0,1	14,5	14,2	17,0	22,4	17,1	19,7	17,7	7,3	9,5	54,7	7,6	12,5	52,6
14.06.2010	L1	2,0	27,2	12,8	0,5	15,9	12,6	19,5	25,0	18,3	22,2	18,8	7,4	9,4	55,4	7,7	11,9	51,4
15.06.2010	L1	2,6	44,3	11,1	0,3	15,2	13,7	19,9	24,4	19,7	22,1	19,4	7,3	10,2	55,7	7,6	14,4	52,6
21.06.2010	L1	1,6	37,1	15,5	0,4	16,5	11,3	20,7	26,6	18,7	23,6	25,9	7,2	9,8	60,5	7,5	13,1	55,8
22.06.2010	L1	2,4	47,6	18,8	0,4	17,8	13,6	24,1	29,6	20,7	26,8	19,3	7,3	9,4	39,2	7,5	11,6	50,5
21.07.2010	L1	0,2	21,2	8,0	0,4	27,1	21,5	24,1	28,7	29,4	26,4	22,0	7,0	6,3	68,5	7,0	7,3	65,0
22.07.2010	L1	0,6	15,4	5,6	0,4	23,4	22,4	25,1	29,1	24,1	27,1	25,2	7,1	7,5	116,8	7,3	9,0	122,6
23.07.2010	L1	0,8	11,6	16,9	1,5	20,4	20,2	22,0	23,5	22,2	22,7	22,0	7,3	8,2	94,4	7,4	9,9	104,5
24.07.2010	L1	1,6	38,4	16,9	0,4	18,7	18,5	25,2	26,7	22,4	26,0	23,2	7,5	9,0	72,9	7,5	11,6	63,5
25.07.2010	L1	2,3	27,4	5,6	0,2	18,7	16,6	18,8	27,9	24,7	23,3	22,0	7,4	7,7	58,4	7,5	10,2	56,1
26.07.2010	L1	2,6	17,0	1,6	0,1	18,3	15,1	19,2	27,3	21,1	23,2	20,7	7,4	7,8	52,7	7,5	10,2	50,5
27.07.2010	L1	2,6	47,6	4,0	0,1	21,0	16,7	22,8	28,5	25,4	25,6	23,3	7,5	8,1	57,4	7,8	13,1	53,3
28.07.2010	L1	2,8	27,9	1,4	0,1	18,9	18,0	20,8	23,9	23,9	22,3	21,6	7,5	7,7	46,5	7,5	11,2	53,5
29.07.2010	L1	2,9	37,9	1,4	0,0	18,1	15,9	22,7	28,8	23,1	25,8	22,6	7,5	7,1	37,3	7,9	11,1	37,4
04.08.2010	L1	0,5	19,8	3,1	0,2	19,2	16,3	21,2	25,9	19,0	23,6	20,6	7,3	8,1	96,7	7,7	8,8	77,9
05.08.2010	L1	0,7	12,4	6,3	0,5	18,2	14,5	16,9	24,6	18,7	20,7	18,6	7,0	8,1	105,0	7,2	10,2	73,2
06.08.2010	L1	1,2	27,0	7,1	0,3	20,3	16,3	19,6	28,6	22,6	24,1	21,8	7,0	8,1	98,7	7,3	10,4	105,7
07.08.2010	L1	1,6	29,2	7,1	0,2	21,6	14,8	24,1	31,4	24,4	27,8	23,7	7,2	7,9	80,0	7,5	10,5	69,5
08.08.2010	L1	2,1	15,3	7,1	0,5	21,7	18,6	21,0	29,5	23,8	25,3	23,2	7,3	7,3	63,6	7,5	10,4	52,1
09.08.2010	L1	2,5	14,1	17,9	1,3	19,0	16,1	20,2	24,4	18,9	22,3	19,9	7,3	8,4	108,6	7,4	11,2	65,0
11.08.2010	L1	2,4	19,7	4,1	0,2	20,8	19,2	18,9	26,0	22,0	22,5	21,5	7,3	8,1	80,4	7,5	10,1	72,4
12.08.2010	L1	2,7	11,2	12,9	1,2	19,0	16,3	17,4	26,3	19,3	21,8	19,8	7,4	8,0	75,7	7,5	9,7	83,7
13.08.2010	L1	3,4	22,6	2,9	0,1	20,8	16,4	21,7	29,0	22,3	25,3	22,3	7,5	7,4	28,6	7,5	8,1	25,9
14.08.2010	L1	3,6	13,3	2,9	0,2	19,2	17,5	19,0	26,3	18,9	22,6	20,4	7,5	7,1	0,7	7,5	8,0	0,8
15.08.2010	L1	3,7	8,8	2,9	0,3	19,5	17,7	20,7	23,1	19,6	21,9	20,3	7,5	6,7	0,8	7,5	7,8	0,9
20.08.2010	L1	0,5	23,5	14,2	0,6	20,6	15,0	20,1	28,6	22,2	24,3	21,5	7,3	4,0	201,7	7,5	6,4	125,4
21.08.2010	L1	1,0	20,0	14,1	0,7	21,8	16,2	26,9	31,2	22,7	29,0	24,2	7,3	5,4	192,6	7,6	8,5	159,3
22.08.2010	L1	1,5	19,0	14,2	0,7	20,8	17,5	26,1	27,6	20,4	26,8	22,9	7,3	5,2	133,9	7,5	8,7	261,0
23.08.2010	L1	2,1	10,0	4,4	0,4	20,3	17,9	19,4	21,9	20,4	20,7	19,9	7,5	7,1	403,1	7,6	8,5	122,5
24.08.2010	L1	2,2	40,9	17,9	0,4	18,2	17,6	23,6	28,2	17,8	25,9	21,8	7,5	5,3	551,4	7,8	11,2	512,2
30.08.2010	L1	1,6	10,3	3,7	0,4	13,1	12,0	13,3	18,0	13,5	15,6	14,2	7,5	5,9	104,5	7,6	9,0	94,2
07.09.2010	L1	2,3	30,4	2,4	0,1	16,9	11,4	15,3	24,3	16,9	19,8	17,0	7,6	5,7	102,1	7,7	6,8	87,7
08.09.2010	L1	2,5	12,1	8,9	0,7	17,7	13,2	14,1	21,5	18,3	17,8	16,8	7,6	5,1	107,0	7,7	6,5	79,4
09.09.2010	L1	3,0	3,6	1,5	0,4	16,4	15,3	14,3	16,1	16,0	15,2	15,4	7,5	4,8	127,1	7,5	5,4	131,6
10.09.2010	L1	3,1	19,6	4,5	0,2	17,8	15,7	18,6	23,8	19,3	21,2	19,3	7,5	4,7	137,1	7,6	5,6	136,6

11.09.2010	L1	3,4	19,1	4,5	0,2	19,7	14,6	18,6	28,8	19,6	23,7	20,4	7,5	4,5	128,2	7,7	5,5	90,2
12.09.2010	L1	3,6	17,3	4,5	0,3	18,7	14,8	18,7	28,9	18,3	23,8	20,2	7,5	4,7	160,1	7,7	6,2	107,1
13.09.2010	L1	3,9	19,7	11,2	0,6	16,4	15,7	20,0	23,9	16,8	21,9	19,1	7,6	5,3	177,5	7,7	5,7	153,9
15.09.2010	L1	5,3	45,3	14,2	0,3	15,0	14,4	20,9	27,6	15,7	24,3	19,7	7,6	5,1	142,7	7,7	6,1	128,6
21.12.2010	L1	0,3	1,1*	3,2	2,8*	-4,4	10,1	9,4	9,9	9,7	9,6	9,8	7,2	11,1	176,0	7,3	12,5	159,3
22.12.2010	L1	0,5	1,8*	4,9	2,7*	-0,5	9,7	9,9	9,4	10,0	9,6	9,7	7,3	10,7	162,9	7,3	13,1	169,6
03.01.2011	L1	0,3	5,6	-1,5	-0,3	4,9	16,9	15,3	19,0	17,8	17,2	17,3	7,9	9,5	98,8	7,5	12,2	83,2
05.01.2011	L1	0,2	4,3	2,9	0,7	0,6	13,5	14,8	18,3	14,2	16,6	15,2	7,4	10,2	144,0	7,3	12,8	148,4
06.01.2011	L1	0,3	0,9*	2,0	2,4*	5,0	16,0	17,2	18,9	17,7	18,1	17,5	7,5	9,4	132,4	7,5	9,6	141,1
10.01.2011	L1	0,4	4,1	3,2	0,8	4,8	17,4	15,9	13,9	11,5	14,9	14,7	6,8	10,2	165,5	6,9	10,6	175,2
11.01.2011	L1	0,5	11,4	0,8	0,1	3,9	10,8	11,9	17,3	16,8	14,6	14,2	7,1	10,3	171,1	7,2	11,3	159,8
12.01.2011	L1	0,5	2,6	2,8	1,1	7,1	16,6	16,9	16,9	17,1	16,9	16,9	7,2	9,7	174,6	7,2	10,0	187,2
13.01.2011	L1	0,6	1,0	-1,1	-1,1	9,0	17,6	17,4	16,4	17,4	16,9	17,2	6,8	7,8	170,6	7,1	8,6	184,9
17.01.2011	L1	0,8	1,3	-1,5	-1,1	12,6	17,8	17,9	18,6	17,9	18,2	18,0	6,6	7,7	157,0	6,7	8,3	179,0
18.01.2011	L1	0,7	1,5	-1,7	-1,2	9,4	17,3	17,4	17,7	17,2	17,5	17,4	7,1	7,9	116,1	7,3	8,5	125,9
19.01.2011	L1	0,7	3,7	-0,5	-0,1	6,4	17,2	16,7	17,4	16,9	17,0	17,0	7,1	7,8	119,0	7,3	8,6	131,4
20.01.2011	L1	0,6	2,8	1,8	0,6	1,2	16,5	14,1	15,4	15,5	14,7	15,4	7,3	8,3	118,4	7,3	9,7	124,0
30.07.2011	L1	2,7	17,2	1,7	0,1	16,8	19,4	20,1	22,0	19,9	21,0	20,3	6,6	8,9	88,3	6,6	8,8	90,4
31.07.2011	L1	1,4	5,5	1,9	0,3	16,2	19,7	20,0	20,1	19,8	20,1	19,9	6,7	9,0	87,9	6,8	9,0	89,0
01.08.2011	L1	0,7	7,0	7,3	1,1	18,1	19,9	20,2	21,4	21,5	20,8	20,7	6,6	9,0	87,4	6,7	9,0	89,6
02.08.2011	L1	1,2	44,6	16,7	0,4	22,8	21,5	21,5	26,3	20,3	23,7	22,4	6,8	6,8	56,5	6,7	8,0	70,5
03.08.2011	L1	2,0	48,6	23,8	0,5	23,3	16,1	23,5	28,0	18,9	25,7	21,6	7,0	4,0	9,6	7,2	5,2	5,1
04.08.2011	L1	2,5	15,8	8,3	0,5	21,7	17,4	18,5	19,4	19,2	18,9	18,6	7,0	3,6	1,0	7,0	4,6	1,0
05.08.2011	L1	2,8	31,0	4,0	0,1	20,7	16,6	18,5	25,1	18,8	21,8	19,7	7,1	3,6	1,0	7,1	5,8	1,0
08.08.2011	L1	3,9	24,5	20,4	0,8	14,6	15,4	17,8	18,7	16,4	18,2	17,1	7,2	4,9	1,0	7,4	5,6	1,0
09.08.2011	L1	3,7	22,7	-5,7	-0,3	14,5	15,3	17,4	18,7	15,7	18,1	16,8	7,1	4,4	9,5	7,2	5,1	6,3
10.08.2011	L1	3,9	24,2	16,6	0,7	13,7	14,8	18,0	19,3	15,2	18,7	16,8	7,1	4,0	18,3	7,2	5,4	13,3
23.03.2012	L1	0,5	46,5	4,2	0,1	10,6	3,2	8,6	20,3	10,4	14,4	10,6	6,9	8,2	64,5	7,0	11,7	65,4
24.03.2012	L1	0,8	50,9	10,1	0,2	9,0	4,4	8,8	15,2	7,5	12,0	9,0	7,4	9,4	52,4	7,5	15,8	53,5
12.04.2012	L1	0,4	17,9	1,8	0,1	7,9	11,9	13,8	20,9	16,1	17,4	15,7	7,4	10,4	74,2	6,9	14,5	80,9
13.04.2012	L1	0,6	23,5	2,8	0,1	6,8	13,2	17,1	18,9	17,3	18,0	16,6	6,7	9,5	78,2	6,8	11,9	84,0
18.04.2012	L1	1,3	30,2	7,4	0,2	10,1	11,6	11,7	23,6	19,1	17,6	16,5	6,8	9,8	58,4	6,9	13,3	76,9
09.05.2012	L1	4,1	36,2	22,8	0,6	17,3	12,6	18,7	21,4	16,7	20,0	17,3	6,7	4,6	116,9	6,9	6,7	124,7
10.05.2012	L1	4,6	12,9	-2,9	-0,2	17,7	12,2	17,3	23,0	18,4	20,1	17,7	6,7	4,6	119,3	6,7	7,0	119,5
14.05.2012	L1	5,8	38,5	19,4	0,5	11,4	2,1	10,6	19,6	13,1	15,1	11,4	6,7	5,3	102,1	6,9	7,7	107,6
19.04.2011	L2	0,5	21,9	14,0	0,6	15,3	8,6	14,1	22,0	14,4	18,0	14,8	7,0	7,5	103,5	7,0	9,3	100,0
20.04.2011	L2	1,1	33,7	31,0	0,9	15,6	8,0	14,9	33,0	25,0	24,0	20,2	7,0	6,5	108,2	7,0	8,4	109,3
21.04.2011	L2	1,8	31,6	17,2	0,5	17,2	19,9	27,5	30,3	21,0	28,9	24,7	7,1	8,5	107,8	7,1	8,7	99,0
22.04.2011	L2	1,9	27,9	1,4	0,1	17,5	14,7	23,2	29,6	21,6	26,4	22,3	7,0	8,4	103,8	7,1	13,4	108,1
24.04.2011	L2	2,1	30,4	11,9	0,4	17,1	14,6	20,4	29,0	23,8	24,7	21,9	6,9	7,5	103,2	7,0	7,6	101,4
25.04.2011	L2	2,1	31,3	11,9	0,4	15,3	14,1	19,9	29,1	23,3	24,5	21,6	6,9	8,7	101,7	7,0	8,7	101,8
26.04.2011	L2	2,5	41,6	2,6	0,1	15,5	13,6	20,1	30,8	23,5	25,5	22,0	6,9	8,6	104,6	7,9	10,0	93,4
27.04.2011	L2	1,4	30,5	12,4	0,4	15,5	14,4	20,6	31,5	16,7	26,0	20,8	6,8	6,4	115,8	7,0	8,2	123,8
28.04.2011	L2	1,9	26,2	9,5	0,4	13,9	11,4	14,5	28,4	20,2	21,4	18,6	6,7	6,5	111,3	6,9	9,5	114,1
29.04.2011	L2	3,0	38,9	11,1	0,3	14,9	13,7	19,8	30,3	19,3	25,1	20,8	6,5	4,2	113,5	6,8	5,8	99,3
30.04.2011	L2	3,0	53,5	15,3	0,3	13,0	9,9	21,3	28,8	18,1	25,0	19,5	6,6	4,2	90,2	6,6	6,2	110,5
01.05.2011	L2	3,0	61,4	17,5	0,3	10,4	9,2	23,0	28,9	13,8	26,0	18,7	6,7	5,2	95,9	6,7	8,1	125,5

02.05.2011	L2	4,8	53,3	21,7	0,4	7,7	7,7	20,2	21,3	11,2	20,8	15,1	6,6	5,1	107,2	6,7	9,1	113,3
03.05.2011	L2	4,8	40,9	16,7	0,4	7,3	7,0	24,9	24,0	11,7	24,5	16,9	6,5	4,7	116,7	6,7	7,4	140,6
04.05.2011	L2	6,1	42,2	17,4	0,4	8,7	9,2	20,2	27,1	15,7	23,6	18,0	6,7	5,0	105,0	7,1	8,0	99,1
05.05.2011	L2	6,7	43,7	12,2	0,3	10,0	12,2	22,9	29,4	22,7	26,1	21,8	6,8	4,8	98,7	7,1	7,3	102,2
06.05.2011	L2	7,6	37,9	8,7	0,2	15,0	11,6	23,1	31,5	23,5	27,3	22,4	6,7	4,1	103,5	7,0	5,7	115,3
07.05.2011	L2	7,6	37,0	8,5	0,2	18,6	12,8	24,5	31,5	24,1	28,0	23,2	6,9	4,7	102,8	7,1	5,2	101,3
08.05.2011	L2	7,6	48,4	11,1	0,2	18,9	13,6	26,2	31,5	24,1	28,9	23,9	7,0	4,3	98,8	7,2	4,4	104,0
09.05.2011	L2	8,4	35,0	6,2	0,2	20,8	15,2	27,7	31,0	23,5	29,4	24,4	6,9	4,9	101,3	7,0	4,2	102,3
10.05.2011	L2	8,5	33,7	6,8	0,2	20,4	15,5	25,9	34,2	27,3	30,0	25,7	6,7	4,2	101,9	7,1	6,1	110,5
11.05.2011	L2	4,4	26,9	12,7	0,5	16,8	16,6	18,6	30,1	26,2	24,3	22,8	6,7	4,3	102,6	6,7	5,1	111,1
01.08.2011	L2	0,6	7,0	2,8	0,4	16,8	19,3	19,9	22,1	19,9	21,0	20,3	6,8	8,5	109,7	6,9	8,6	109,6
02.08.2011	L2	0,9	44,6	17,3	0,4	16,2	19,6	19,9	20,2	19,8	20,0	19,9	6,8	5,1	100,9	6,7	6,8	106,9
03.08.2011	L2	1,5	48,6	19,7	0,4	18,1	19,7	20,0	21,4	21,5	20,7	20,7	6,9	4,1	114,2	6,9		119,3
04.08.2011	L2	1,8	15,8	8,3	0,5	22,8	21,5	21,5	26,5	20,9	23,8	22,6	7,0	8,4	111,4	6,9	10,5	116,1
05.08.2011	L2	2,3	31,0	9,0	0,3	23,3	16,2	23,2	28,2	19,1	25,7	21,7	7,0	7,2	100,2	7,2	12,2	100,1
08.08.2011	L2	3,6	24,5	16,2	0,7	21,7	17,5	18,6	19,5	19,4	19,0	18,7	7,0	6,9	108,2	7,0	10,3	113,4
09.08.2011	L2	4,0	22,7	16,1	0,7	20,7	16,6	18,4	25,2	19,2	21,8	19,9	7,0	7,2	105,6	7,0	9,0	101,5
10.08.2011	L2	4,4	24,2	15,9	0,7	21,0	16,1	19,0	23,6	18,2	21,3	19,2	7,0	7,7	109,3	7,0	8,6	102,0
23.03.2012	L2	0,9	46,5	14,1	0,3	10,6	12,6	21,9	32,1	14,7	27,0	20,3	7,1	7,9	44,4	7,5	12,8	46,0
24.03.2012	L2	1,8	50,9	15,4	0,3	9,0	8,0	19,6	28,1	10,5	23,9	16,5	6,8	6,9	51,2	7,2	16,1	58,6
12.04.2012	L2	2,0	17,9	23,1	1,3	7,9	7,1	10,4	18,0	11,4	14,2	11,7	6,9	7,5	110,5	7,0	10,5	119,8
13.04.2012	L2	2,8	23,5	6,6	0,3	6,8	9,1	13,6	15,8	12,7	14,7	12,8	7,1	5,6	102,2	7,1	12,0	104,6
18.04.2012	L2	0,7	30,2	10,2	0,3	10,1	7,5	8,1	21,4	14,1	14,8	12,8	7,2	6,9	125,2	7,4	9,3	136,7
19.04.2012	L2	1,2	45,5	5,9	0,1	11,2	6,9	16,4	28,1	14,3	22,2	16,4	6,9	5,2	124,7	7,4	13,9	137,9
20.04.2012	L2	1,8	42,2	15,9	0,4	11,0	9,3	19,0	27,1	15,9	23,0	17,8	7,0	6,6	119,6	7,4	12,7	138,8
21.04.2012	L2	2,3	28,5	5,6	0,2	9,5	8,9	17,8	19,8	10,3	18,8	14,2	7,2	4,5	115,3	7,9	8,4	94,0
22.04.2012	L2	2,7	20,1	6,6	0,3	6,7	5,4	11,2	19,3	10,4	15,2	11,6	7,2	5,3	111,8	7,4	8,2	112,7
23.04.2012	L2	3,4	29,2	18,5	0,6	9,3	5,4	13,4	22,8	16,5	18,1	14,5	7,3	5,4	111,6	7,6	12,5	98,9
09.05.2012	L2	5,4	36,2	17,3	0,5	17,3	15,1	21,6	26,8	18,5	24,2	20,5	7,2	4,9	182,6	7,6	11,5	220,3



Supplementary figure 2 Broken stick model (red solid line) to determine the important components (blue line with dots) of the principal component analysis for the 17 parameter of the basic data set in supplementary table 1. Only the first and second principal components were used for further analysis of the key parameter for the biomass productivity.