Engineering in Life Sciences

Eng. Life Sci. **2014**, *00*, 1–9 www.els-journal.com

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Research Article

Phototrophic growth of *Arthrospira platensis* in a respiration activity monitoring system for shake flasks (RAMOS®)

Optimizing illumination is essential for optimizing the growth of phototrophic cells and their production of desired metabolites and/or biomass. This requires appropriate modulation of light and other key inputs and continuous online monitoring of their metabolic activities. Powerful noninvasive systems for cultivating heterotrophic organisms include shake flasks in online monitoring units, but they are rarely used for phototrophs because they lack the appropriate illumination design and necessary illuminatory power. This study presents the design and characterization of a photosynthetic shake flask unit, illuminated from below by warm white light-emitting diodes with variable light intensities up to 2300 μmol m^{-2} s^{-1}. The photosynthetic unit was successfully used, in combination with online monitoring of oxygen production, to cultivate *Arthrospira platensis*. In phototrophic growth under continuous light and a 16 h light/8 h dark cycle (light intensity: 180 μmol m^{-2} s^{-1}), the oxygen transfer rate and biomass-related oxygen production were −1.5 mmol L^{-1} h^{-1} and 0.18 mmol O_2 g_x^{-1} h^{-1}, respectively. The maximum specific growth rate was 0.058 h^{-1}, during the exponential growth phase, after which the biomass concentration reached 0.75 g L^{-1}.

Keywords: *Arthrospira platensis* / Cyanobacteria / LED / Oxygen transfer rate / Photobiotechnology

Received: December 20, 2013; *revised:* April 11, 2014; *accepted:* April 30, 2014

DOI: 10.1002/elsc.201300156

1 Introduction

Phototrophic organisms produce diverse metabolites that are difficult or impossible to obtain by cultivating heterotrophs, ranging from dietary supplements to chemicals used in the pharmaceutical, cosmetics, and biofuel sectors [1, 2]. Thus, photobiotechnology is an emerging field in sustainable production; diverse techniques have been developed for cultivating phototrophic cells, and the efficiency of photobioreactors is improving [3–7]. However, better understanding of the relationships among light, growth, and production would improve large-scale production. There is also increasing demand for systems for cultivating phototrophic cells that supply CO_2 and light efficiently and flexibly while allowing continuous online monitoring of key metabolic parameters such as oxygen production.

Shake flasks, which are often used in small-scale investigations, can efficiently supply carbon dioxide and oxygen, but irradiating all the cells equally in a culture can be problematic because the intensity of penetrating light decreases with culture depth due to absorption of the photons by the phototrophic cells. Thus, the growth of cells varies inversely with their distance from the light source.

Several techniques for calculating photosynthetic activity from oxygen evolution, which is believed to be the most accurate approach [8], have been published, primarily for cultures of microalgae or cyanobacteria [9, 10]. However, these have yielded variable results due to differences in the methodology applied [11].

The Respiration Activity MOnitoring System® (RAMOS®, HiTec Zang GmbH, Herzogenrath, Germany) is a powerful instrument for parallel screening using shake flasks while monitoring total oxygen and carbon dioxide transfer (OT and CT, respectively) and corresponding rates (OTR, CTR). The system comprises eight measuring flasks with attached sensors that measure pressure and oxygen partial pressure, together with six standard cotton-plugged shake flasks for reference sampling. The reference flasks are aerated passively in the surrounding incubator. The measuring flasks are aerated with the same aeration rate

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Abbreviations: OLED, organic light-emitting diode(s); **PAR**, photosynthetically active radiation; **PBR**, photobioreactor; **PCC**, Pasteur Culture Collection; **RAMOS®**, Respiration Activity MOnitoring System®

(10 mL/min) via particular tubing during the rinsing phase, in order to ensure comparable conditions. Furthermore, they are closed at regular intervals to measure changes in pressure and oxygen partial pressure resulting from the metabolic activity of the cells, to calculate OTR and CTR. The use of RAMOS to cultivate heterotrophic organisms such as yeast, bacteria, and plant cell lines is well described [12–18].

In one published study, RAMOS was used to cultivate a photosynthetically active *Wolffia australiana* plant cell line [19]. Fluorescent lamps were placed above the shake flasks and the OTR was −0.1 mmol L^{-1} h^{-1} under continuous illumination and −0.05 mmol L^{-1} h^{-1} under light/dark cycles. The drawbacks of this set-up were that the cell suspension was shaded by the sensor rack above the flasks and only a single fixed light intensity was applied. In several other studies, the shake flasks were illuminated with light-emitting diode (LED) from below [20, 21], but not in combination with online (oxygen and carbon dioxide) monitoring.

No satisfactory system has been previously described that combines adequate illumination of the shake flasks with adjustable light intensity for application in incubator shakers with orbital shaking motion and online monitoring systems like RAMOS. Thus, in the study presented here, a system with these features was developed. It includes a lighting unit designed to illuminate all flasks evenly from below, with adjustable and reproducible light intensities including tightly controlled light/dark cycles if required. In tests of the system *Arthrospira platensis* cells were cultivated and the results demonstrate that it provides the desired capacities.

2 Materials and methods

2.1 Microorganism and media

Arthrospira platensis PCC 9108 was obtained from the Pasteur Culture Collection (PCC, Paris, France). Cells were maintained in wide neck Erlenmeyer flasks in an incubator shaker with orbital shaking motion (Minitron, Infors HT, Bottmingen/Basel, Switzerland, shaking diameter 25 mm) and a photosynthetic unit of six fluorescent lamps (Grolux 15 W, Osram Sylvania, Danvers, MA, USA) above the flasks, supplying an average light intensity of 25 μmol m^{-2} s^{-1} at the surface of the cell suspension. Flasks were incubated at 30°C and 110 rpm for 14 days with a light/dark cycle of 16 h/8 h.

Zarrouk medium was used with the following concentrations of nutrients; macroelements (g L^{-1}): $NaNO_3$ 2.5, K_2SO_4 1.0, NaCl 1.0, $MgSO_4 \cdot 7\ H_2O$ 0.2, $CaCl_2 \cdot 2\ H_2O$ 0.04, $NaHCO_3$ 13.61, Na_2CO_3 4.03, K_2HPO_4 0.5; microelements from premixed 200-fold concentrated stock solution (μg L^{-1}): $ZnSO_4 \cdot 7\ H_2O$ 5, $MnSO_4 \cdot 4\ H_2O$ 10, H_3BO_3 50, $Co(NO_3)_2 \cdot 6\ H_2O$ 5, $Na_2MoO_4 \cdot 2\ H_2O$ 5, $CuSO_4 \cdot 5\ H_2O$ 0.025, $FeSO_4 \cdot 7\ H_2O$ 0.0035.

All nutrients were dissolved separately in deionized water and mixed in the specified order to prevent precipitation. The microelement solution was added to the macroelement solution and made up to final volume with deionized water (final pH 9.1). The medium was sterilized by filtration (cellulose acetate, 0.45 μm, Sartorius, Göttingen, Germany). For photomixotrophic cultivation, a sterile filtered stock solution of glucose was added to a final concentration of 1.5 g L^{-1}.

All flasks were inoculated with 10 v/v% of vital cell suspension in the linear growth phase.

2.2 Cultivation system

2.2.1 Respiration Activity MOnitoring System

The Respiration Activity MOnitoring System (RAMOS, HiTec Zang GmbH, Herzogenrath, Germany) was used as previously described [17] with the following additional steps.

Flasks were wrapped separately in aluminium foil to exclude ambient light and light from adjacent LED, and a flask to humidify inlet air (for measurement flasks only) was mounted in front of the gas supply block. Narrow neck Erlenmeyer flasks with cotton-wool plugs were used as reference flasks for OD sampling to ensure comparability to the measurement flasks.

2.2.2 Light sources

Illumination was provided by a set-up of warm white LED (CRI 90+PowerBar LED, Nichia, Tokushima, Japan), as described in detail in the results section.

Light intensity was measured with a Li-190 Quantum Sensor (Li-Cor Biosciences GmbH, Bad Homburg vor der Höhe, Germany) for monitoring light intensity (PAR 400–700 nm).

2.3 Cultivation

The LED were installed, adjusted to deliver the desired light intensity and regime, and the RAMOS flasks were prepared with appropriate plugs, separately wrapped in aluminium foil, sterilized by autoclaving (121°C, 20 min, 1 bar g) and dried at 100°C to remove condensed water. The flasks were then mounted into the RAMOS and left shaking (110 rpm, shaking diameter 25 mm) for at least 1 h to equilibrate the temperature (30°C) after which the pressure test was accomplished to ensure the tightness of the flasks and the oxygen sensors were calibrated.

The method of preinoculation was applied as described previously [17]. The cell suspension was adjusted to a dry biomass concentration of 0.1 g L^{-1}, and the desired volume (30 mL) was pipetted into each measuring and reference flask. All flasks were then again mounted into the RAMOS and measurement proceeded with a rinsing/measuring phase ratio of 10/20 min. OD was measured by sterile sampling.

2.4 Analyses

2.4.1 Transfer rates

The OTR and CTR of the cultures were calculated automatically by the RAMOS using equations previously described [17].

2.4.2 Specific growth rate

The specific growth rate (μ) of each culture during the exponential growth phase was calculated from both changes in the biomass concentration (μ_x, Eq 1). and the OTR (μ_{OTR}, Eq 2). using the following equations:

$$\mu_x = \frac{\ln \frac{C_{x2}}{C_{x1}}}{\Delta t} \quad (1)$$

$$\mu_{OTR} = \frac{OTR \times Y_{\frac{C_x}{O_2}}}{C_x} \quad (2)$$

with biomass concentration C_x in g L^{-1}, measuring time interval Δt in h, oxygen transfer rate OTR in g_{O2} L^{-1} h^{-1}, yield coefficient for biomass per oxygen $Y_{\frac{C_x}{O_2}} = 0.5076\ g_x\ g_{O2}^{-1}$ for *A. platensis* [2].

2.4.3 Dry biomass concentration

The dry biomass concentration (C_x) of the cell suspensions was calculated from their OD at 750 nm, determined using a Beckman DU 640 UV/VIS-spectrophotometer (Beckman-Coulter, Brea, CA, USA). The relationship between OD and dry biomass concentration was determined in advance as: $C_x = OD_{750\,nm} \cdot 0.82$ g L^{-1}.

3 Results

3.1 Lighting unit for RAMOS

3.1.1 Design of illumination

The light source was mounted below the flasks to prevent shading by the rack of sensors mounted on top (Fig. 1A) and maximize the uniformity of the illumination throughout the flasks. Space was left between the mounting of the flasks and the LED to allow insertion of dimming, diffusing or filtering material as required. Each LED panel consisted of a row of six circular warm white LED (Fig. 1B and C) mounted on a stainless steel plate with thermal conduction adhesive to dissipate heat through the base. This set up represents one LED panel.

Warm white LED were chosen because they illuminate across nearly all the absorption maxima of the photosynthetic pigments (Fig. 1D): chlorophylls (430–450 nm, 640–690 nm), phycobiliproteins (500–650 nm), and carotenoids (400–500 nm). Bacterial chlorophylls can also be excited in the range of 400–750 nm. However, if ultraviolet or infrared illumination is required, an additional or different set of LED could be installed.

3.1.2 Distribution of light intensity in the shake flasks

The distribution of light emitted by one LED panel (Fig. 1C) over the base area of the shake flasks with a selected range of potentiometer settings was measured at the bottom of an empty shake flask at various points directly above and between the LED positions (Fig. 2). The distribution of light across the bottom of the flasks was very uneven, especially at high light intensities because LED are punctual light sources. For example, at the highest potentiometer setting (Fig. 2A), the light intensities at the center of the flask bottom ($x = 0$; $y = 0$), directly above one LED in the circle of five LED, and selected points between the LED were 4300 μmol m^{-2} s^{-1}, 2500 μmol m^{-2} s^{-1}, and

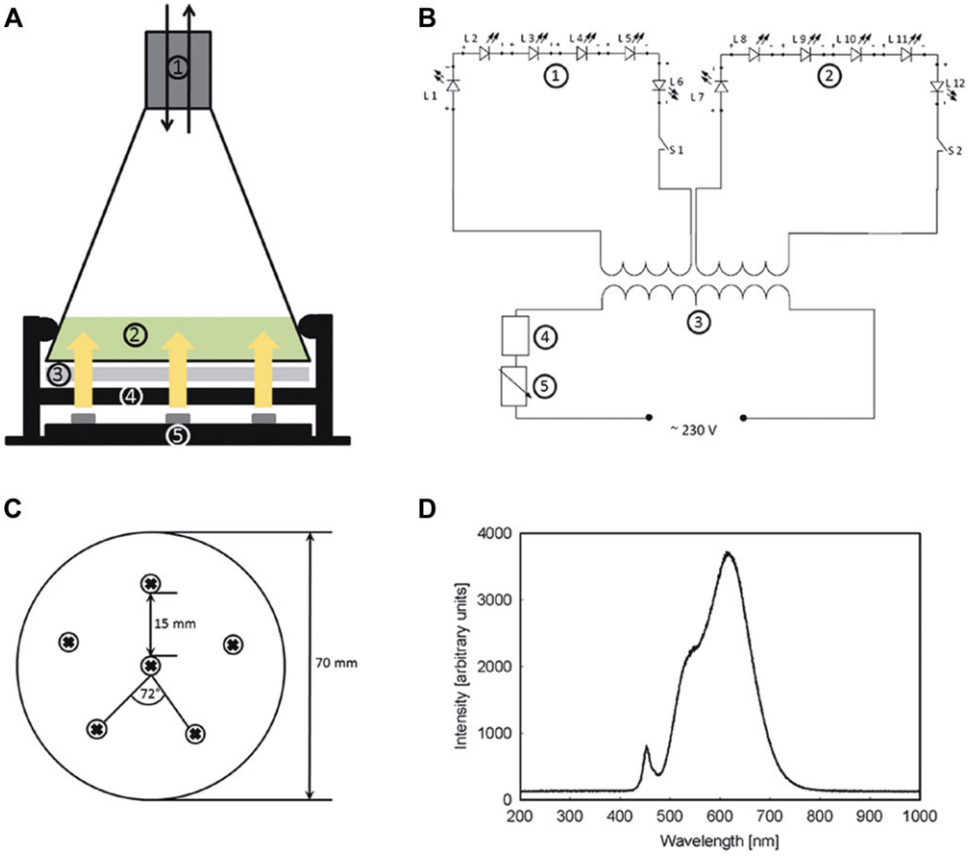

Figure 1. Design of the lighting unit for the shake flask system: (A) side view of flask arrangement, mounting and LED panel with (1) oxygen and pressure sensor with gas exchange unit, (2) phototrophic cell suspension, (3) diffusor/dimming/filter plate, (4) mounting for flask, and (5) LED panel; (B) circuit diagram of the power supply of two connected LED panels with (1) LED panel 1, (2) LED panel 2, (3) transformer, (4) digital display, and (5) potentiometer; (C) top view of LED panel arrangement (positions of LED are cross-marked); and (D) wavelength spectrum of the warm white LED.

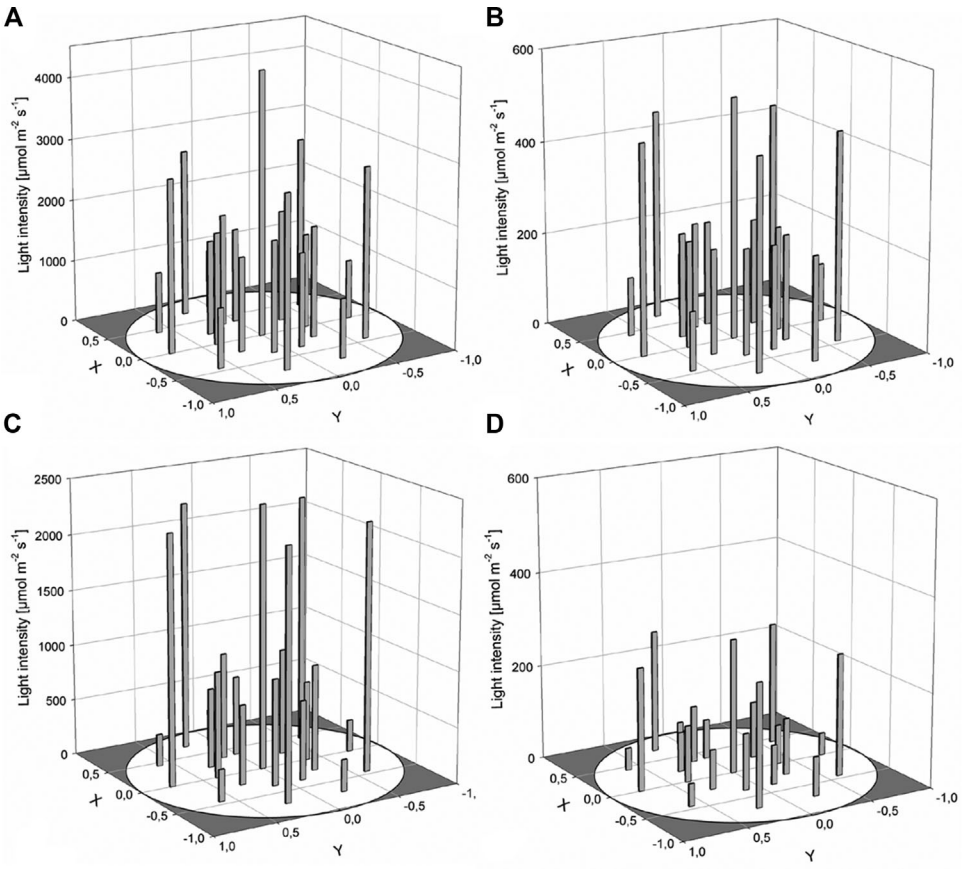

Figure 2. Distribution of light intensity (μmol m^{-2} s^{-1}) emitted by the circular array of six LED in one panel over the flask base (white area) with center point ($x = 0$; $y = 0$), (A) potentiometer setting maximum, no dimming or diffuser material inserted; (B) potentiometer setting minimum, no dimming or diffuser material inserted; (C) potentiometer setting maximum, 2 mm acrylic glass as diffuser material inserted; and (D) potentiometer setting minimum, 2 mm acrylic glass as diffuser material inserted.

900–1500 μmol m^{-2} s^{-1}, respectively. With the lowest potentiometer setting (Fig. 2B), the corresponding light intensities were 520, 450, and 130–230 μmol m^{-2} s^{-1}, respectively. However, when 2 mm thick acrylic glass was used as a diffuser the light distribution was more even, and other diffuser materials might offer further improvement (Fig. 2C and D).

3.1.3 *Range of light intensity*

Two LED panels can be used to illuminate two flasks simultaneously, independently from the other flasks, using a potentiometer connected to a digital display to monitor light intensity. The range of potential photon flux densities can be varied from 0 to an average of 2300 μmol m^{-2} s^{-1}, as illustrated by the examples in Table 1. The semiconductor in the LED structure requires a specific voltage to emit photons, which limits the minimum potentiometer setting. However, the photon flux density can be further reduced, if required, by using dimming materials such as acrylic glass, paper or fabric.

Thus, the lighting unit can provide a wide range of light intensities to meet the requirements of selected phototrophic organisms, corresponding to the range from sunlight to dim light under water (for comparison, the solar constant 1368 W m^{-2} corresponds to 6000 μmol m^{-2} s^{-1} [22]). Light intensities above 2000 μmol m^{-2} s^{-1} are rarely beneficial for cultivation because they inhibit or even damage the photosystems of most cells. For example, *Arthrospira* spp. are photoinhibited by light intensities exceeding 1300 μmol m^{-2} s^{-1} [22].

Table 1. Mean light intensity in μmol m^{-2} s^{-1} provided by one LED panel of the lighting unit with various potentiometer settings and possible dimming/diffuser materials.

Potentiometer setting	Dimming/diffuser material	Mean light intensity (μmol m^{-2} s^{-1})
Maximum	None	2300
Maximum	White acrylic glass 2 mm	1500
Maximum	White acrylic glass 4 mm	1000
Maximum	Commercial printing paper	750
Minimum	None	340
Minimum	White acrylic glass 2 mm	190
Minimum	White acrylic glass 4 mm	130
Minimum	Commercial inkjet paper	90

In addition to varying the light intensity by adjusting the voltage and/or using diffuser or dimming materials, it is also possible to insert filter material to block or enhance particular wavelengths from the normal spectrum of the LED. Alternatively, the LED panels can be changed, if LED with different spectra are required. The lighting system can thus be used to provide a broad range of light intensities and spectra to cultivate diverse phototrophs.

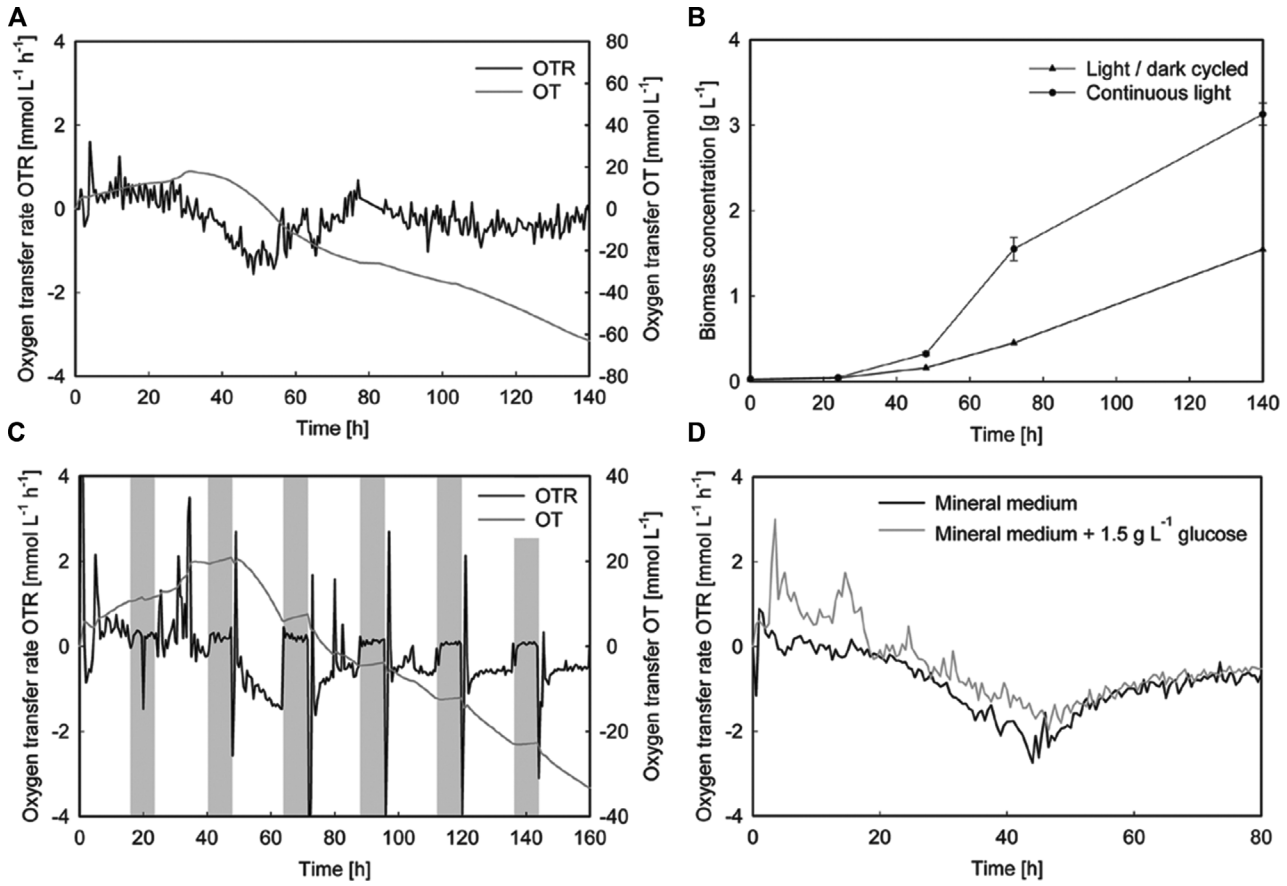

Figure 3. Cultivation of *A. platensis* PCC 9108 in RAMOS with the lighting unit using Zarrouk medium with a light intensity of 180 μmol m^{-2} s^{-1} and temperature 30°C: (A) oxygen transfer rate (OTR) in mmol L^{-1} h^{-1} (solid black line) and oxygen transfer (OT) in mmol L^{-1} (solid gray line) under continuous light; (B) biomass concentration in g L^{-1} under continuous light (circles) and light/dark cycles (triangles); (C) oxygen transfer rate (OTR) in mmol L^{-1} h^{-1} (solid black line) and oxygen transfer (OT) in mmol L^{-1} (solid gray line) with a light/dark regime of 16 h/8 h, gray bars indicate dark cycles; and (D) oxygen transfer rate (OTR) in mmol L^{-1} h^{-1} during photomixotrophic cultivation in medium supplemented with 1.5 g L^{-1} glucose (solid gray line) and reference with mineral medium (solid black line).

3.1.4 *Light/dark cycles*

Light/dark cycles ranging from a few minutes to several hours can be established using a timer that controls the lighting system's power supply. It is planned that this will be done via the RAMOS control unit, permitting light/dark cycles spanning seconds or even milliseconds to study the effects of flashing light on phototrophic organisms, which are also of great interest [23–26].

3.1.5 *Stability of light, temperature, pressure, and oxygen sensor readings during operation of RAMOS with the lighting unit*

The stability of the system was tested over 200 h under cultivation conditions (light intensities of 180 and 1500 μmol $m^{-2}s^{-1}$, shaking at 110 rpm, 30°C). No changes in either light intensity or the temperature in the incubator housing were detected during this time, demonstrating the constancy of the light and effectiveness the RAMOS cooling system with the lighting unit fully powered. In addition, no differences in pressure or oxygen readings were observed when the lighting unit was operational compared to values obtained in the dark (data not shown). However, the temperature of the liquid in the flasks increased with increasing light intensity, as discussed in detail later.

3.2 Cultivation of *A. platensis* PCC 9108 in RAMOS under different light regimes

3.2.1 *Cultivation under continuous light*

After setting up the lighting unit, the RAMOS system was initially used to cultivate the cyanobacterium *A. platensis* PCC 9108 under continuous light. This organism was chosen because it is a model system with broad applications, including use of its whole biomass as a nutritional supplement and production of specific compounds such as phycocyanins and polyunsaturated fatty acids for use in cosmetics or pharmaceuticals [2, 27–29].

The cells were able to grow fully photosynthetically under the spectrum of the implemented warm white LED. The OTR (Fig. 3A) increased to a mean value of 0.5 mmol L^{-1} h^{-1} in the first 30 h, then decreased to a minimum of −1.5 mmol L^{-1} h^{-1} after 48 h before finally increasing again to −0.5 mmol L^{-1} h^{-1}. The CTR (data not shown) hardly deviated from zero, as

expected. No carbon dioxide was produced or consumed during the process; the cells utilized dissolved hydrogen carbonate from the alkaline medium (pH 9) and carbon dioxide produced during respiration was immediately dissolved in the medium and not emitted as gas.

Under these conditions 18 mmol L^{-1} of oxygen was initially consumed and 80 mmol L^{-1} was subsequently produced (Fig. 3A) equivalent to a biomass-related oxygen production of 0.18 mmol O_2 $g_{biomass}^{-1}$ h^{-1}. Biomass in the flasks increased nearly exponentially from 0.03 g L^{-1} to 0.33 g L^{-1} in the first 48 h, and finally reached with linear increment 3.13 g L^{-1} after 140 h (Fig. 3B). OT slowed between 48 h and 80 h then declined linearly, correlating with the constant OTR and linear cell growth. This pattern indicates that the organisms predominantly respired during the lag phase (the first 30 h), after which they were photosynthetically active and produced oxygen, first exponentially and then linearly.

However, strong fluctuations were observed in the OTR signal. These may have been caused by variations in humidity and temperature inside the flasks, to which the sensors are extremely sensitive. Therefore, the accuracy of the measurements of pressure and oxygen signals may have been affected.

The peak in the OTR was probably due to the relationship between light intensity and cell density in the liquid medium. At the low initial concentration all cells are able to absorb sufficient light. As the density of cells increases light becomes limiting due to mutual shading. Further, as the concentration of the pigments within the cells increases, the individual cells absorb more light. Thus, although the supply of light is constant, most of it is absorbed before it can penetrate far into the liquid. In the present study, with an incident light intensity of 180 μmol m^{-2} s^{-1}, this occurred at a cell density of 0.75 g L^{-1}, corresponding to the minimum OTR.

This can be formally described using the Lambert–Beer law which relates the attenuation of light intensity over path length to the absorption properties of the material through which it travels. Based on this law the cell suspension, with an extinction coefficient (ε) of 1.36 L g^{-1} cm^{-1} (determined empirically) and a depth (d) of 1 cm, absorbs already 64% of the incident light (intensity 180 μmol m^{-2} s^{-1}) after 48 h. Thus, the growth curve slowed from exponential to linear, with a productivity of 0.018 g L^{-1} h^{-1}.

The specific growth rate μ_x was calculated to 0.058 h^{-1} for the first 48 h (Eq. 1), assuming exponential growth during this period. The specific growth rate μ_{OTR} in the first 48 h was 0.053 h^{-1} (Eq. 2), which is not significantly different from μ_x, indicating that the OTR measurement was accurate.

3.2.2 Cultivation under a 16 h/8 h light/dark regime

A light/dark regime of 16 h/8 h was used to study the effect of light cycles on the photosynthetic rate of the cultivated cells. The OTR and OT readings clearly showed that they were photosynthetically active and produced oxygen during the light phases, but not the dark phases, as expected (Fig. 3C).

During the dark periods, no oxygen was produced, the OTR was close to zero and OT was constant. During the light periods the OTR followed the same pattern as under continuous light. In the third light cycle (after 64 h of cultivation) the OTR decreased to a minimum of −1.5 mmol L^{-1} h^{-1} when biomass concentration reached 0.2 g L^{-1} (Fig. 3B), within the range observed during continuous illumination. The OTR increased during the exponential growth phase, and plateaued at −0.5 mmol L^{-1} h^{-1} (Fig. 3C) in the linear phase (Fig. 3B).

However, fluctuations in the OTR were detected at the beginning of light cycles, indicating interference with the measurement, probably due to temperature increases in the flasks when the LED were switched. It is also possible that excess light energy was converted into heat as a result of the protection mechanisms in the photosynthetic machinery of the cells [30]. This warming affected at least one measurement phase, until the thermostat readjusted. This corroborated the sensitivity of the pressure and oxygen sensors, and shows that adjustments are needed to avoid fluctuations at the beginning of light cycles. For example, measurements could be omitted at the beginning of light phases.

3.2.3 Cultivation under photomixotrophic conditions

Cultures were also grown under photomixotrophic conditions to test the influence of additional energy sources on OTR measurements (Fig. 3D). The mineral medium was therefore supplemented with 1.5 g L^{-1} glucose, which *A. platensis* can utilize [2, 31, 32].

Initially the OTR of the photomixotrophic culture was higher than that of phototrophic culture, probably due to higher respiration rate induced by the glucose. The subsequent decrease in OTR was smaller, reaching a minimum of −1.5 mmol L^{-1} h^{-1} after 48 h compared with −2.0 mmol L^{-1} h^{-1} for the phototrophic cultures. However, the final OTR was identical (−0.5 mmol L^{-1} h^{-1}). In contrast to the previous experiments (with continuous illumination), the minimum OTR in both culture types was reached at cell densities of more than 1 g L^{-1} (data not shown). However, a higher cell density was used for inoculation in these experiments, complicating direct comparison with the OTR under the continuous light treatment. Light limiting conditions may have been reached earlier and the glucose was not consumed completely, the residual concentration being 1 g L^{-1}.

4 Discussion

4.1 Lighting unit

This study demonstrated the effectiveness of the lighting unit for illuminating the shake flasks in the RAMOS. However, the results also indicate several possible improvements. Firstly, as observed in the reported experiments, all light sources, including LED, inevitably introduce thermal energy into incubation systems. The additional heat increases evaporation in the shake flasks and the water vapor absorbs light and converts it into heat, a process known as a greenhouse effect with water vapor as the most important greenhouse gas [33]. A temporary solution to counter these temperature increases would be to decrease the incubator temperature (which is only possible for cultivations with continuous light). More permanent refinements would include using a cooling system with a higher capacity and faster response times. The dissipation of heat over the shaking platform should also

Table 2. Specific growth rate μ in h^{-1} (μ_x and μ_{OTR} calculated from biomass and oxygen transfer rate OTR, respectively), minimum oxygen transfer rate OTR_{min} in mmol L^{-1} h^{-1}, total oxygen transfer OT_{total} in mmol L^{-1}, and biomass related oxygen production (BROP) in mmol O_2 g_x^{-1} h^{-1} obtained from cultivation of *Arthrospira platensis* PCC 9108 in RAMOS with the lighting unit under different light regimes with light intensity of 180 μmol m^{-2} s^{-1}.

Light and nutrition regime	OTR_{min} (mmol L^{-1} h^{-1})	OT_{total} (mmol L^{-1})	BROP (mmol O_2 g_x^{-1} h^{-1})	μ_x / μ_{OTR} (h^{-1})
Continuous phototrophic	−1.5	80	0.18	0.058 / 0.053
16 h / 8 h Light / dark cycled phototrophic	−1.5	50	0.20	0.057 / 0.054
Continuous photomixotrophic	−1.5	60	–[a]	–[b]

[a] Calculation not possible due to combined photoautotrophic and heterotrophic growth.
[b] Insufficient data.

be improved. Passive cooling could be achieved by increasing the distance between the LED panel and the flasks to improve air circulation or using metal cooling elements. Another option would be to install a fan at the bottom of the incubator housing in addition to the existing fan at the top to promote circulation of air and thus removal of heated air at the bottom of the incubator nearby the lighting unit.

In addition, to prevent fluctuations at the beginning of light cycles, the measurement and light regimes could be linked, so that the light is only switched on at the beginning of the RAMOS rinsing phase and not during the measurement phase. The rinsing phase could also be extended to delay measurement until the temperature reaches equilibrium or measurement phases where light was switched could be omitted.

The distribution of light over the base of the flasks is very uneven because LED are punctual light sources. Diffuser materials were inserted, but more effective material could be identified or other light sources used, such as organic light-emitting diodes (OLED) that provide more even light distribution. However, this would involve a reduction in light intensity because the OLED currently available cannot produce the same light intensity as high power LED. Thus, they would only be viable alternatives for cultivations under low light intensities. A further anticipated refinement is to enable light/dark intervals in milliseconds in order to study the effects of light flashes described in the literature [23–26].

4.2 Cultivation of *Arthrospira platensis*

The results demonstrate that *A. platensis* PCC 9108 can be successfully cultivated in RAMOS using warm white LED. The effects of several light regimes were examined, including continuous illumination and light/dark cycles of 16 h/8 h. OTR and OT were used to distinguish the different phases of phototrophic and photomixotrophic growth. The OTR and OT readings together with specific growth rates calculated from the biomass and OTR measurements under each of the light and nutrition regimes are summarized in Table 2.

A similar pattern of OTR was detected under each light and nutritional regime tested. There was an initial lag phase as the cells adapted from the low intensity fluorescent light used during subcultivation to the high-intensity LED light. Respiratory rates exceeded photosynthetic rates in this phase, as demonstrated by positive OTR measurements. In the next phase OTR decreased to negative values, as growth and photosynthetic activity increased, until the cell density reached a critical value where cells could not absorb sufficient light for exponential growth (Table 2). After this point OTR plateaued, reflecting the linear growth phase which is a common phenomenon in the cultivation of phototrophs [3,7].

The minimum OTR of −1.5 mmol L^{-1} h^{-1} observed in this study was in the same order of magnitude as values for heterotrophic plant cell lines such as *Azadirachta indica* (neem), *Salvia fruticosa* (sage) and *Helianthus annuus* (sunflower) [13,17,18]. However, it was an order of magnitude higher than reported minima for a line of *Wolffia australiana* (duckweed) cells, grown both phototrophically and photomixotrophically [19], and a photomixotrophically grown plant cell line of *H. annuus* [18]. This probably reflects the different growth rates and energy sources (carbon or light) of the respective systems. Phototrophic plant cell lines such as duckweed have a doubling time of 1–3 days [34] compared to 9–12 h for *Arthrospira*, and their oxygen evolution rates differ accordingly.

Arthrospira platensis had a biomass-related oxygen production rate of 0.2 mmol O_2 g_x^{-1} h^{-1} under continuous light and light/dark cycles with a light intensity of 180 μmol m^{-2} s^{-1}. The calculated specific growth rate μ was within the range reported in the literature for similar light intensities (0.051 h^{-1} [35]; 0.048 h^{-1} [2]; 0.058 h^{-1} [22]). However, the estimates may have been influenced by differences between the measurement flasks (used for OTR and OT determinations) and reference flasks (used to measure biomass). The pigment content of the cells (chlorophyll a and phycobiliproteins) may also be influenced by the warm white LED spectrum, and this possibility warrants investigation.

4.3 Concluding remarks

The OTR readings fluctuated substantially, more than measurements reported elsewhere [14, 19]. This may be because the RAMOS oxygen and pressure sensors are highly sensitive to changes in temperature and heat from the LED, which probably increases the temperature and humidity in the shake flasks.

Effects of the volume of culture in the flasks on mixing and gas transfer need further investigation. To improve comparability, the preculture should also be cultivated in RAMOS or at least with the same lighting unit to eliminate effects of differences in physiological states pre- and postinoculation (includ-

ing differences in pigment contents and photosynthetic parameters [36, 37]), and allow the cells to adapt to LED light. The mounting could also be improved by putting the light source in adhesive foils similar to those used for mounting the shake flasks. The lighting unit is also suitable for use in other orbital shaking incubator systems for cultivating phototrophic organisms under controlled conditions, and these possibilities warrant further investigation. In addition, mirrors or reflecting material could be installed below the LED to augment illumination to the base of the flasks. A gas mixing station could be added to supply CO_2 in order to extend the technique to organisms that grow in neutral or acidic media rather than alkaline media like *Arthrospira*.

Once all of these refinements have been introduced, it will be necessary to conduct further tests with *A. platensis* and other organisms to calculate oxygen production rates over the whole range of light intensities from compensation point to saturation and photoinhibition. Knowledge of these variables is necessary for understanding and optimizing phototrophic cultivation.

In summary, the lighting unit is a useful tool for cultivating *Arthrospira* and other phototrophic organisms under controlled light conditions in the RAMOS and similar shake flask systems with simultaneous online monitoring of growth, photosynthesis, and oxygen production.

Practical application

Photobiotechnology is an emerging field in bioprocessing, based on the exploitation of phototrophic organisms to produce compounds with diverse applications (inter alia) in the food, pharmaceutical and energy sectors. Numerous kinds of photobioreactors have been developed to cultivate these organisms and produce desired compounds. The most common include shake flasks and various instruments for monitoring key performance parameters online. This paper presents a system for illuminating flasks incubated in an orbital shaker system. Its utility is demonstrated in a set-up with a Respiration Activity MOnitoring System (RAMOS) to measure oxygen production by *Arthrospira*, an important cyanobacterium. The presented set-up can be used to optimize light-dependent parameters for the cultivation and practical exploitation of phototrophic cells.

The authors thank Tim Lauterbach and Robert Stange for soldering support, Christian Löser and Susanne Steudler, all Chair of Bioprocess Engineering, Technische Universität Dresden, for productive discussions, and the Department of Biology, Technische Universität Dresden, for kindly providing the light sensor. The work was financed by the Free State of Saxony, the European Regional Development Fund (project no. 100065054), and the Central Innovation Program of the Federal Ministry of Economics and Technology (BMWi; project no. KF2049811MD3).

The authors have declared no conflict of interest.

Nomenclature

CT	[mmol L^{-1}]	carbon transfer
CTR	[mmol L^{-1} h^{-1}]	carbon transfer rate
C_x	[g L^{-1}]	dry biomass concentration
d	[cm]	depth in cm
OT	[mmol L^{-1}]	oxygen transfer
OTR	[mmol L^{-1} h^{-1}]	oxygen transfer rate
OTR_{min}	[mmol L^{-1} h^{-1}]	minimal oxygen transfer rate
OT_{total}	[mmol L^{-1}]	total oxygen transfer
rpm	[min^{-1}]	revolutions per minute
ε	[L g^{-1} cm^{-1}]	extinction coefficient
Δt	[h]	measurement interval
$Y_{\frac{C_x}{O_2}}$	[g_x $g_{O2}{}^{-1}$]	yield coefficient for biomass per oxygen
μ	[h^{-1}]	specific growth rate
μ_x	[h^{-1}]	specific growth rate calculated on biomass
μ_{OTR}	[h^{-1}]	specific growth rate calculated on oxygen transfer rate

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