MitoFit Preprints 2021.5 doi:10.26124/mitofit:2021-0005
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Experimental Communication

Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by High-Resolution PhotoRespirometry

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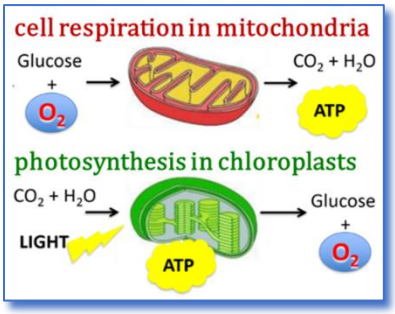
Posted Online 2021-04-30

Abstract



The bioenergetic crosstalk between mitochondria and chloroplasts plays a key role in maintaining metabolic integrity and controlling metabolite production for growth and regulation of cell concentration. Dark respiration and photosynthesis were measured in the green alga *Chlamydomonas reinhardtii* at varying oxygen concentrations and three cell concentrations using the NextGen-O2k with the PhotoBiology Module. Maximum net photosynthesis at a light intensity of 350 µmol·s^{-1}·m^{-2} (blue light) was inhibited at hyperoxia by 40 % at oxygen concentrations of 550 to 650 µM. Light-enhanced dark respiration reached a (negative) maximum within 30 to 60 s after light-dark transitions and was 3.5- to 4-fold higher than steady-state dark respiration independent of O_2 concentration in the range of 200 to 650 µM.

Keywords – high-resolution respirometry, photosynthesis, dark respiration, *Chlamydomonas reinhardtii*

High-Resolution PhotoRespirometry and cell culture

High-resolution respirometry based on the Oroboros O2k is extensively applied to the study of mitochondrial physiology in the biomedical field [1,2]. Real-time oxygen flux was measured using the NextGen-O2k, a two-chamber instrument, in growth medium TRIS at 25 °C. Light intensities (blue) were controlled with the PhotoBiology-Module in the range from 0 to 350 µmol·s^{-1}·m^{-2} (Figure 1). Data were recorded by DatLab 7.4.

Algae were grown photoautotrophically in growth medium TRIS (N- and P-nutrient replete) at 25 °C and a light intensity of 100 µmol·s^{-1}·m^{-2} (16:8 h L:D) [3]. Six cultures (N=6) were harvested by centrifugation at 1000 g (10 min) and diluted in TRIS.

1. O_2 flow as a function of the light regime and O_2 concentration

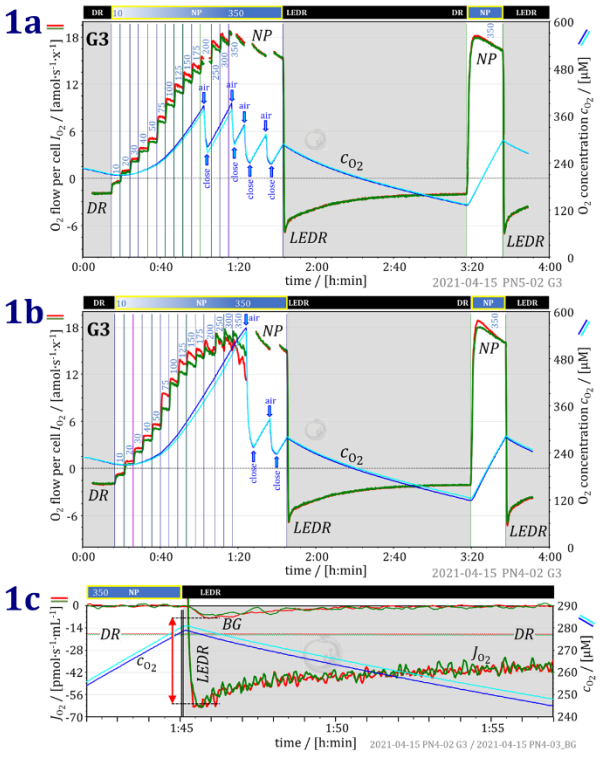


Figure 1. O_2 flow I_{O2} as a function of the light regime and O_2 concentration c_{O2}. Superimposed traces of c_{O2} and I_{O2} in two O2k-chambers. Maximum net photosynthesis *NP* was obtained at light intensities of 300 to 350 µmol·s^{-1}·m^{-2} (vertical numbers).

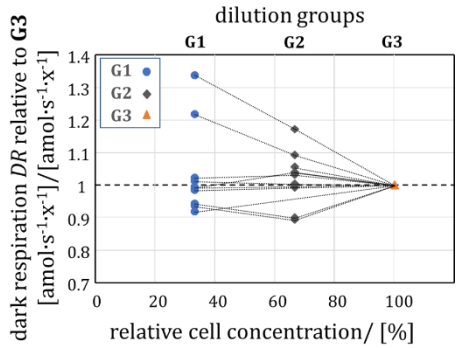
The net O_2 production rate (net photosynthesis *NP*) was stimulated from dark respiration *DR* at normoxia to a maximum by stepwise increments of light intensity (blue light; 10 to 350 µmol·s^{-1}·m^{-2}). The compensation point at zero *NP* was between 10 and 20 µmol·s^{-1}·m^{-2}. Light-enhanced dark respiration *LEDR* was a sharp (negative) maximum of respiration immediately after switching off the light (Figure 1).

1a. The O_2 concentration was prevented from reaching severe hyperoxia by intermittently opening the chambers (arrows, air) and continuing the record of O_2 flow per cell I_{O2} [amol·s^{-1}·x^{-1}] [4].

1b. The O_2 concentration increased in the closed chamber due to *NP*. The decline in maximum *NP* was reversed by lowering the O_2 concentration.

1c. Light-enhanced dark respiration *LEDR* was a sharp (negative) maximum respiratory flux per volume J_{O2} [pmol·s^{-1}·mL^{-1}] at 30-60 s after light-dark transitions. Instrumental background *BG* indicated a small transient disturbance of the O_2 signal by switching off the light, which was accounted for in the background correction for O_2 flux.

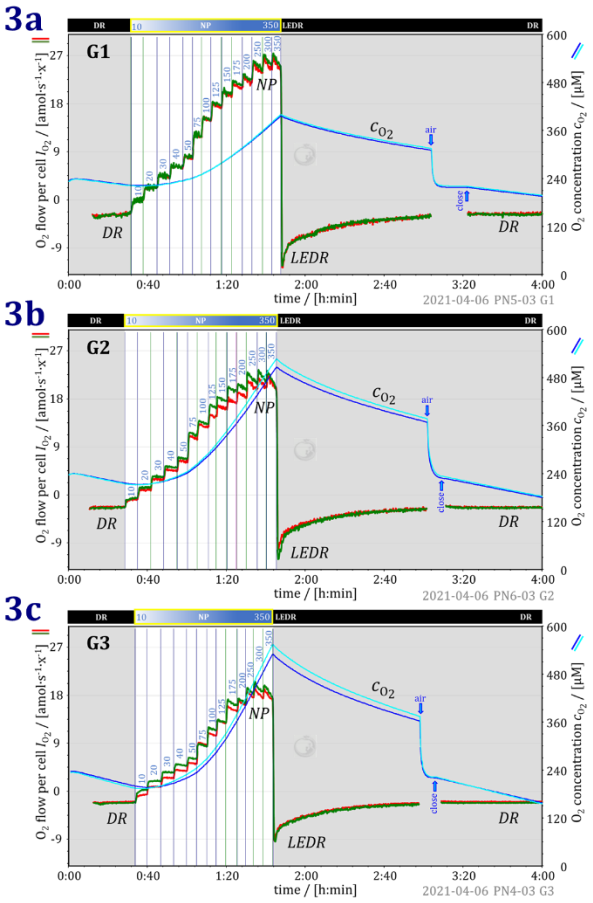
2. Dark respiration



In each of five culture harvests (experimental replica; *N*=5), dilution group **G3** was diluted to **G2**. **G2** was diluted further to **G1**. Cell concentration C_{ce} of **G3** was approximately 9·10^6 x·mL^{-1}. Dark respiration *DR* expressed as O_2 flow per cell [amol·s^{-1}·x^{-1}] was independent of C_{ce}. *DR* is shown relative to *DR* of **G3** (Figure 2). *DR* was measured initially at normoxia simultaneously in two technical repeats of three cell dilutions (*n*=2 repeats × 3 dilution groups; Figure 3).

Figure 2. Dark respiration *DR* measured simultaneously in three cell dilutions, expressed relative to dilution group **G3**.

3. Maximum net photosynthesis as a function of cell concentration and O_2 concentration

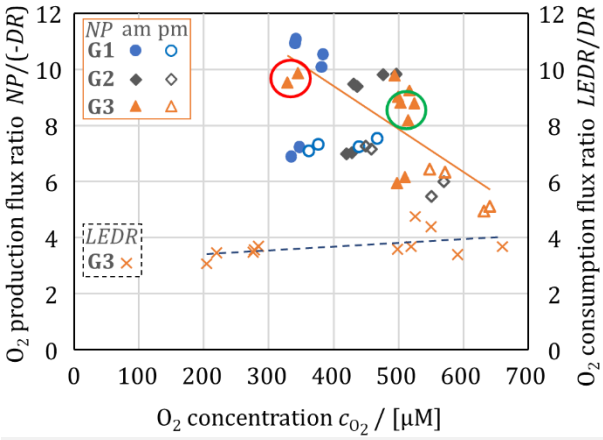


A stepwise increase of light intensity (Figure 3; vertical numbers, 10 to 350 µmol·s^{-1}·m^{-2}) stimulated net photosynthesis *NP* to a maximum while O_2 concentration increased from 220 µM to 400, 520, and 550 µM depending on cell count per volume in the closed reaction chamber (Figure 3; dilution groups **G1** to **G3**).

The lower *NP* capacity at higher cell concentration was caused by hyperoxic inhibition of photosynthesis (Figure 4).

Figure 3. O_2 flow at different cell concentrations (G1 to G3) determines O_2 concentrations at increasing light intensities in the closed chamber. Superimposed traces of oxygen concentration c_{O2} and O_2 flow per cell I_{O2} in two O2k-chambers. Maximum net photosynthesis *NP* was obtained at light intensities of 300 to 350 µmol·s^{-1}·m^{-2} (vertical numbers). *DR* returned to initial levels 2 h after the *LEDR* peak.

4. Oxygen dependence of net photosynthesis and light-enhanced dark respiration



Independent of cell concentration, *NP* was inhibited gradually from normoxia to severe hyperoxia by up to 40 %. There were no consistent differences between measurements in the morning (am) or afternoon (pm; Figure 4).

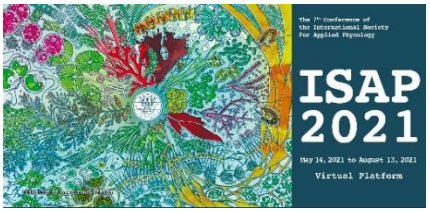
Light-enhanced dark respiration *LEDR* measured at normoxia and hyperoxia was 3.5- to 4-fold higher than *DR*. *LEDR* did not significantly depend on O_2 concentration (Figure 4).

Figure 4. Oxygen dependence of net photosynthesis *NP* and light-enhanced dark respiration *LEDR*. O_2 flux ratios normalized for *DR*. Red and green circles: data from Figure 1a and 1b.

Conclusions

The decline of net O_2 production under hyperoxia was not caused by compensatory light-enhanced photorespiration *LEPR*, if *LEDR* is proportional to *LEPR* [5,6], but by inhibition of photosynthesis at high oxygen concentrations. *LEDR* was 3.5- to 4-fold higher than steady-state dark respiration *DR*. *DR* returned to initial levels 2 h after the *LEDR* peak.

Acknowledgements



Presented at ISAP2021 - https://isap2020-phycology.org/. The hardware and electronics of the NextGen-O2k PB-Module was developed in collaboration with WGT-Elektronik GmbH & Co KG. We thank M. Huete-Ortega for technical support. The NextGen-O2k project has received funding from the European Union's Horizon 2020 research and innovation program under the grant agreement No 859770.

Author contributions

NW and MDM conducted and EG designed the experiment. NW and EG carried out the data analysis and co-wrote the manuscript. All authors commented on and approved the manuscript.

Conflicts of interest

EG is founder and CEO of Oroboros Instruments, Innsbruck, Austria.

Data availability

Original files are available Open Access at Zenodo repository: 10.5281/zenodo.4729616

Abbreviations

C_{ce} count concentration of cells [Mx·mL^{-1}]; c_{O2} amount concentration of oxygen [µM]; *DR* dark respiration; J_{O2} oxygen flux per volume [pmol·s^{-1}·mL^{-1}]; I_{O2} oxygen flow per cell [amol·s^{-1}·x^{-1}]; *LEDR* light-enhanced dark respiration; *NP* net photosynthesis

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