Supplementary Information:
Photosynthetic Carbon Uptake Correlates with Cell Protein Content During Lipid Accumulation in the Microalgae *Chlorella vulgaris* NIES 227

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SUPPLEMENTARY INFORMATION S1 : ESTIMATION OF THE PHOTON FLUX UTILIZED BY THE MICROALGAE DURING OXIMETRIC EXPERIMENTS

It is challenging to estimate the light used by microalgae in the OX1LP-6 (Qubit™, Canada) set-up. Due to light reflexion in the cell water chamber surrounding the experimental chamber, modelling light distribution was deemed to not be possible. Instead, specific calibration measurements were performed to link microalgae biomass light absorption characteristics and light field as measured in the experimental chamber

Material and methods

Calibration of light intensity field

The experimental chamber was filled with serial dilution of a lab-grown *Chlorella vulgaris* NIES 227 test culture. Light absorption of each tested dilution was measured (spectrophotometer Epoch 2, Biotek™, United States) for the wavelengths 880 nm and 683 nm. The light absorbance measured for each tested dilution at these two wavelengths are summarized in Table S1

Table S1: Light absorbance for the wavelengths 880 nm and 683 nm of *Chlorella vulgaris* NIES 227 cultures serially diluted used for the calibration of light field determination in the experimental chamber of OX1LP-6 set-up.

			Absorbance (corrected for blank value)	
		Wavelength (nm)	880	683
	Blank (ultra-pure water)	0	0	0
	32	0.03125	0.08	0.199
	16	0.0625	0.158	0.395
Dilution factor	8	0.125	0.306	0.777
	4	0.25	0.635	1.616
	2	0.5	1.046	3.013
	1	1	1.551	3.569

The experimental chamber filled with microalgal dilutions was lit under at 100 ± 2 µmol·m^{-2}·s^{-1} (as indicated by the constructor software). For each tested dilution, PAR intensity in the experimental chamber was measured on 15 defined points with a light meter ULM-500 (Walz™, Germany) associated with a sensor US-SQS/L (Walz™, Germany). Light measured were carried out in a dark room to avoid interferences with other light source. These defined points were chosen in the following vertical axis of the experimental chamber: front, extreme sides, central, and back (with respect to the source light intensity direction) on three points. The three points measured for each axis were at the bottom and top of the vertical axis, as well as the point of maximal light intensity for a given vertical axis which was estimated to be at a 1/3rd of the total height of the experimental chamber (from the bottom horizontal plan). These points are henceforth referred to as light sampling points and are represented in Figure S1.1. The light sampling points were numbered for identification.
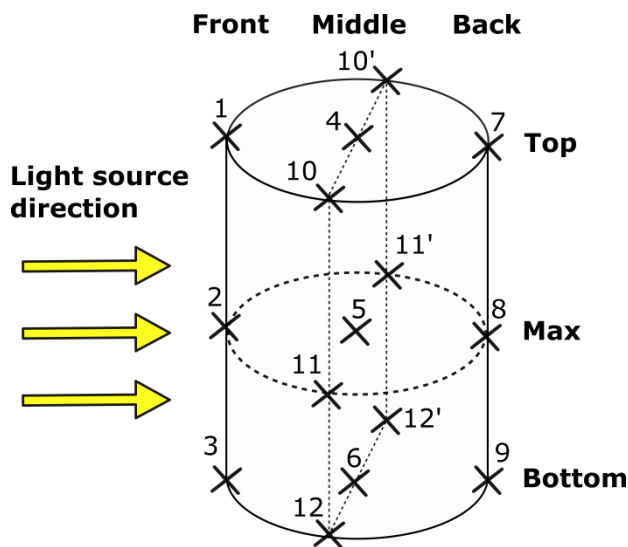

Figure S1.: Graphical representation of the experimental chamber of OX1LP-6 set-up and the sampling points for light intensity (shown as crosses, identified by numbers).

Results

Light intensity according to incident light intensity and microalgal solution light absorbance characteristics

The PAR light intensity measured in the experimental cell was found to correlate well with the microalgal solution light absorbance at 880 nm at every light sampling points. The full result of the measurements are given in Table S2.

Table S2: Light intensity as measured in each sampling point in the experimental chamber according to the dilution of the microalgal culture tested

Light sampling point		1	2	3	4	5	6	7	8	9	10	11	12
	Blank	6	115	51	7	105	0	9	91	29	6	91	39
	32	5	120	52	6	95	0	7	84	25	6	84	34
Dilution factor	16	5	118	57	5	88	0	7	66	24	5	83	35
	8	4.5	111	49	4	70	0	4.5	49	18	4.5	73	33
	4	4	108	50	3	50	0	3	30	11	3	76	29
	2	3.5	102.7	32	1.5	32	0	1.5	12	6	1.5	40	16
	1	3	97	28	0.5	12	0	0.6	4	2.5	1	21	25

Mathematical laws linking local light intensity with the absorbance at 880 nm could therefore be derived from this calibration for each light sampling point. For the sampling point of the front and central vertical axis (points 1,2, and 3) and the sides vertical axis (points 10, 11, 12, 10', 11', and 12'), linear relationships were found as shown in Figure S2.2 for points 1, 2, and 3. The rest of the points were found to follow an exponential-like decrease of light intensity according to the absorbance of the microalgal culture. A mathematical relationship was therefore looked for in the of $I = I_0 \cdot e^{-k_d \cdot A_{880nm}}$ where I_0 is the light intensity measured in the set-up filled with ultra-pure water, A_{880nm} is the absorbance of the microalgal culture for the wavelength 880 nm, and k_d is a sampling point specific extinction coefficient. k_d was fitted by minimizing the sum of squared residuals between the experimental light intensity measured and the predicted value. All results from fitting are shown in Table S1.3
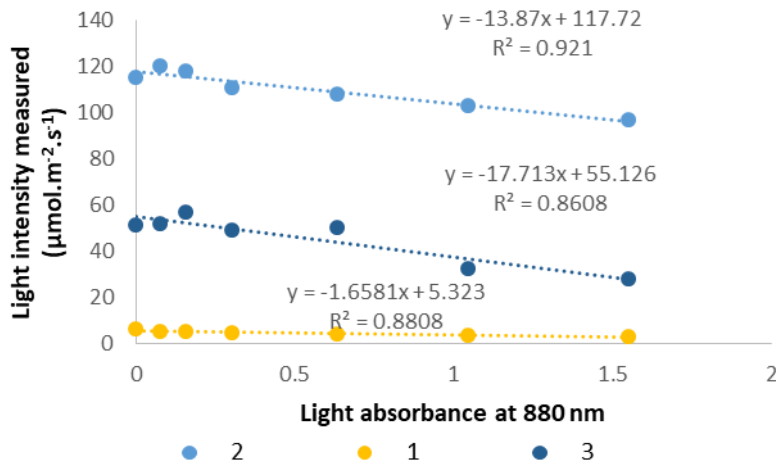
Figure S2: Light intensity in light sampling points 1, 2, and 3 according to the light absorbance at 880 nm of the microalgal solution. Linear regression associated to this data set are also shown.

Table S3: Summary of fitting results and performance for each light sampling point

Sampling point	Slope (if applicable)	Intercept (if applicable)	Extinction coefficient (if applicable)	Coefficient of determination
1	-1.66	5.32		0.954
2	-13.9	117.7		0.939
3	-17.7	55.1		0.884
4			1.59	0.983
5			1.22	0.996
6				
7			1.89	0.981
8			1.87	0.993
9			1.51	0.997
10	-3.46	5.72		0.944
11	-44.0	90.6		0.944
12	-10.8	36.0		0.687

Inferring of the light intensity field in the chamber according to the incident light condition and microalgal solution light absorption characteristics

The light intensity field in the experimental was first determined according to the following steps.

1) The incident light intensity and optical characteristics of the microalgal culture tested are known.

2) Light intensity is first directly determined in the light sampling points from the mathematical formula as developed in previous paragraph.

3) The light intensity field is then computed for the frontal and exit phases of the experimental chamber. To achieve this, both surfaces are partitioned as a mesh of defined number of subdivision (typically 25x25), and light intensity on the meshes are computed by linearly interpolating the value measured for the light sampling points of these surfaces with regards to the cylindrical coordinates of the surfaces.

Likewise, the light intensity field on the central vertical axis is determined from linear interpolation of the measured values on the vertical coordinates of this axis.

4) For any other point of the experimental chamber, light intensity is determined from the linear interpolation the light intensity value of the horizontal projection of this point to 1) the closest outer surface and 2) the central axis of the experimental chamber with regards of the distance to these projections.

5) Finally, the light intensity used by microalgae in a given point of the experimental chamber was assessed as the light extinction coefficient at 683 nm multiplied by the local light intensity field[1].

Code availability

The function used to determine the light field in the experimental chamber of the OX1LP-6 (Qubit™, Canada) experimental cell is available at https://figshare.com/collections/Photosynthetic_carbon_uptake_correlates_with_cell_protein_content_during_lipid_accumulation_in_the_microalgae_Chlorella_vulgaris_NIES_227/6229317[2].

References

(1)   Béchet, Q.; Chambonnière, P.; Shilton, A.; Guizard, G.; Guieysse, B. Algal

Productivity Modeling: A Step toward Accurate Assessments of Full-Scale Algal Cultivation. *Biotechnol. Bioeng.* **2015**, *112* (5). https://doi.org/10.1002/bit.25517.

(2) Chambonniere, P.; Ramírez-Romero, A.; Dimitriades, A.; Sassi, J.-F.; Delrue, F. Photosynthetic Carbon Uptake Correlates with Cell Protein Content during Lipid Accumulation in the Microalgae Chlorella Vulgaris NIES 227. *figshare. Collect.* **2022**. https://doi.org/10.6084/m9.figshare.c.6229317.v1.

SUPPLEMENTARY INFORMATION S2: R™ PACKAGES USED FOR THE PRESENT STUDY

The R™ packages important for the development of this study are cited below.

Neuwirth E (2022). _RColorBrewer: ColorBrewer Palettes_. R package version 1.1-3, <https://CRAN.R-project.org/package=RColorBrewer>.

Garnier, Simon, Ross, Noam, Rudis, Robert, Camargo, Pedro A, Sciaini, Marco, Scherer, Cédric (2021). _viridis - Colorblind-Friendly Color Maps for R_. doi:10.5281/zenodo.4679424 <https://doi.org/10.5281/zenodo.4679424>, R package version 0.6.2, <https://sjmgarnier.github.io/viridis/>.

Hamilton NE, Ferry M (2018). "ggtern: Ternary Diagrams Using ggplot2." _Journal of Statistical Software, Code Snippets_, *87*(3), 1-17. doi:10.18637/jss.v087.c03 <https://doi.org/10.18637/jss.v087.c03>.

Wickham H, Bryan J (2022). _readxl: Read Excel Files_. R package version 1.4.1, <https://CRAN.R-project.org/package=readxl>.

Allaire J, Xie Y, McPherson J, Luraschi J, Ushey K, Atkins A, Wickham H, Cheng J, Chang W, Iannone R (2022). _rmarkdown: Dynamic Documents for R_. R package version 2.16, <https://github.com/rstudio/rmarkdown>.

Xie Y, Allaire J, Grolemund G (2018). _R Markdown: The Definitive Guide_. Chapman and Hall/CRC, Boca Raton, Florida. ISBN 9781138359338, <https://bookdown.org/yihui/rmarkdown>.

Xie Y, Dervieux C, Riederer E (2020). _R Markdown Cookbook_. Chapman and Hall/CRC, Boca Raton, Florida. ISBN 9780367563837, <https://bookdown.org/yihui/rmarkdown-cookbook>.

Wickham H (2016). _ggplot2: Elegant Graphics for Data Analysis_. Springer-Verlag New York. ISBN 978-3-319-24277-4, <https://ggplot2.tidyverse.org>.

Xiao N (2018). _ggsci: Scientific Journal and Sci-Fi Themed Color Palettes for 'ggplot2'_. R package version 2.9, <https://CRAN.R-project.org/package=ggsci>.

Pedersen T (2022). _patchwork: The Composer of Plots_. R package version 1.1.2, <https://CRAN.R-project.org/package=patchwork>.

SUPPLEMENTARY INFORMATION S3: CORRELATION BETWEEN O$_2$ PRODUCTIVITY, AND LIPID AND CARBOHYDRATE CELL QUOTAS
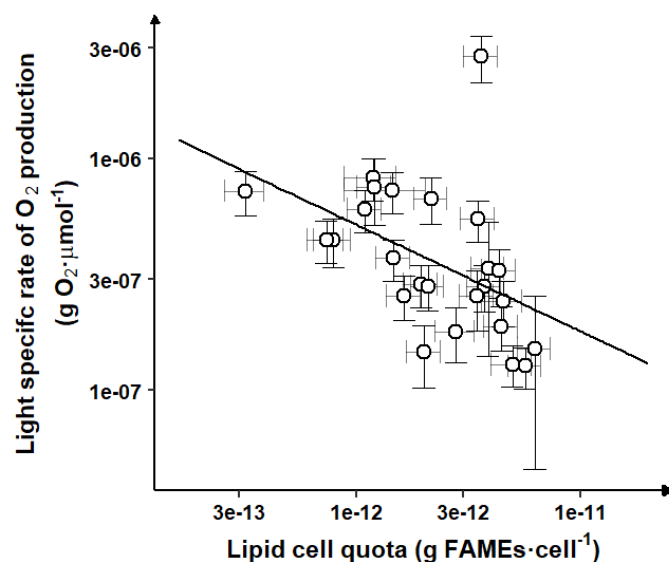

Figure S3 *Chlorella vulgaris* NIES 227 photosynthetic productivity according to the cell lipid quota (o)
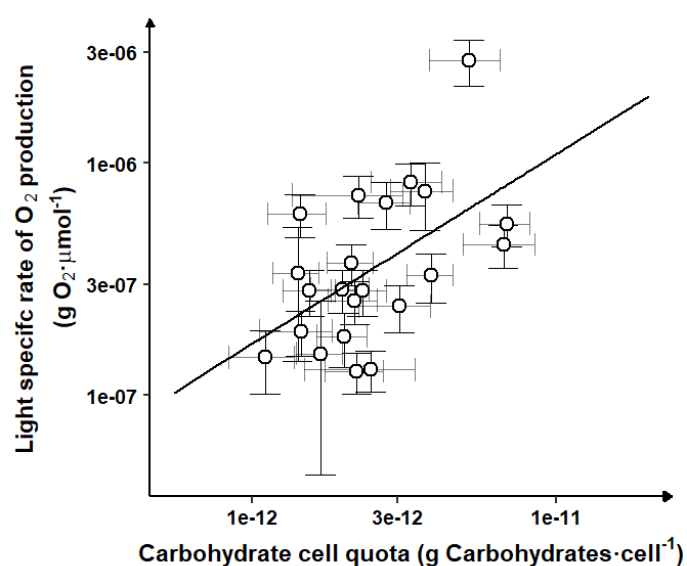

Figure S4 *Chlorella vulgaris* NIES 227 photosynthetic productivity according to the cell carbohydrate quota (o)