

Chlorella vulgaris heterotrophy-to-phototrophy conversion dynamics are mostly independent of light intensity

Supplementary materials

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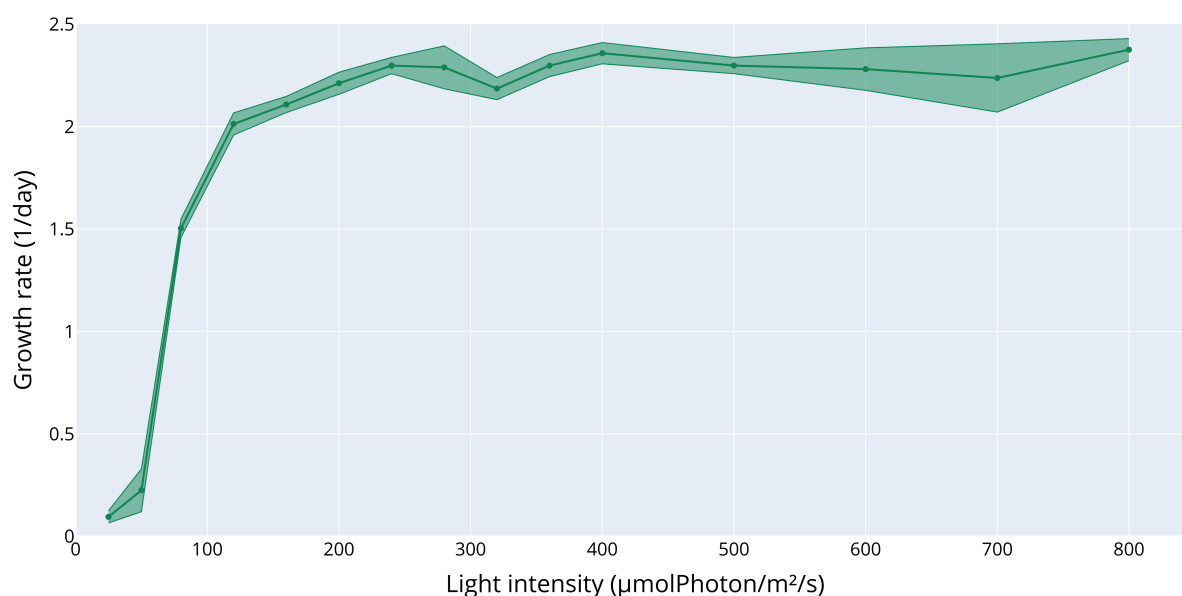
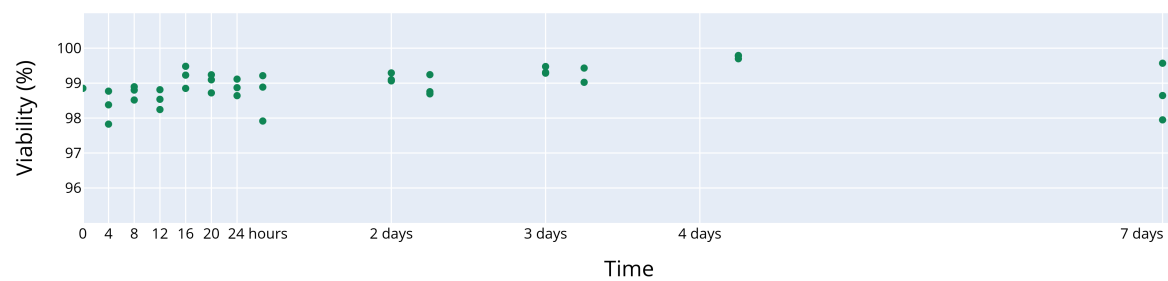
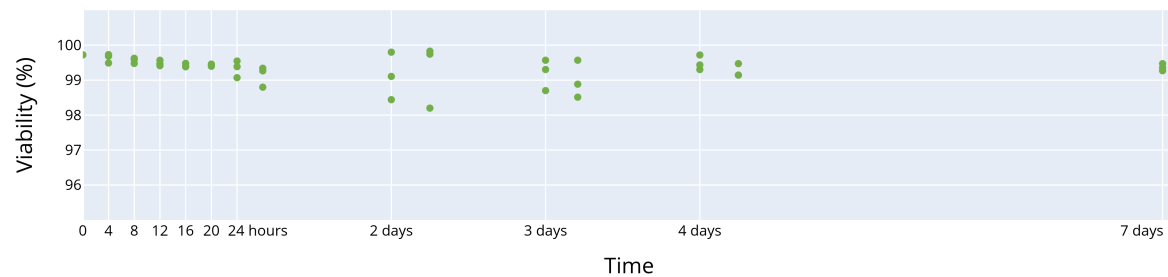


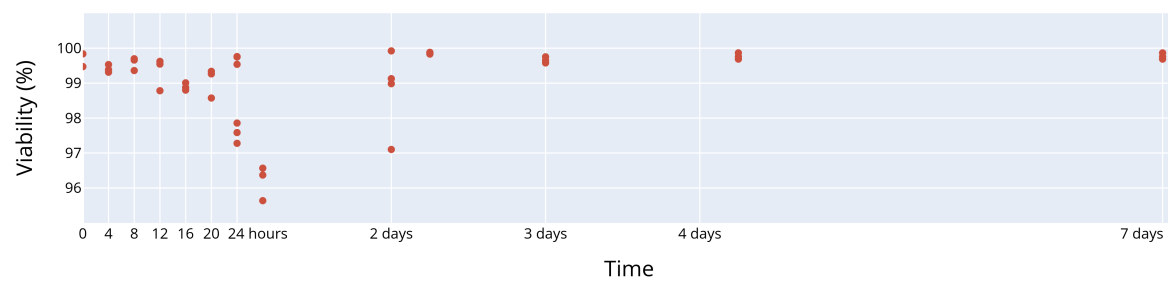
Fig. 1. Growth rate of *Chlorella vulgaris* cells when cultivated in photoautotrophy within the ultrathin flat panel photobioreactors. Obtained in identical conditions as the ones used in this work (125 mL working volume, air with 2.5 % CO₂ sparging at 1.8 vvm, 25 °C controlled by water circulation, glucose-free B3N medium) with the exact same strain 29. Solid line - mean of the three replicates. Shaded area - standard deviation (n=3). Points - Measurements.



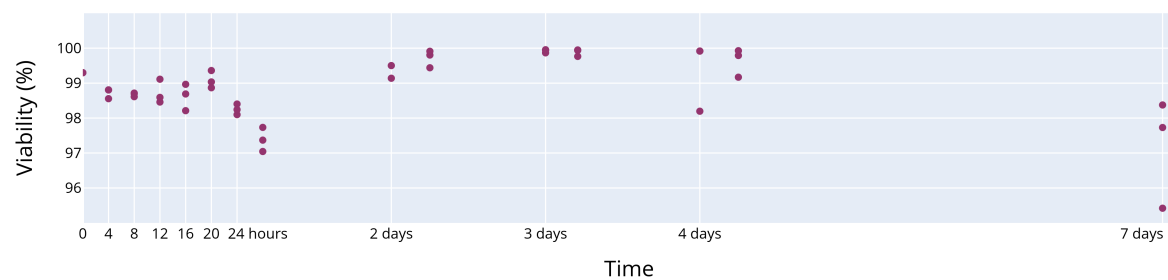
(a) 30 $\mu\text{molPhoton/m}^2/\text{s}$



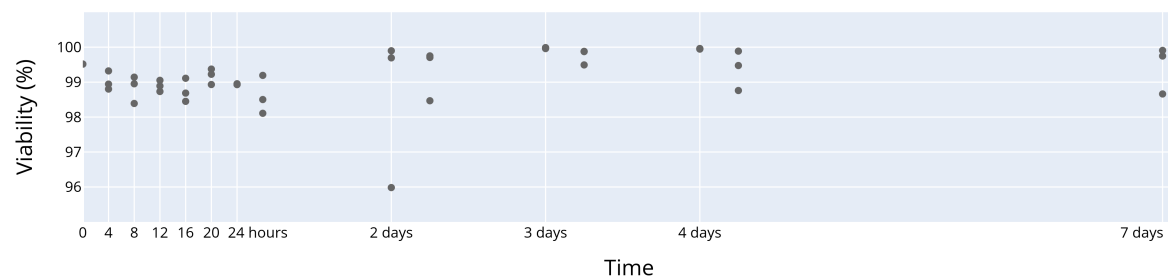
(b) 60 $\mu\text{molPhoton/m}^2/\text{s}$



(c) 300 $\mu\text{molPhoton/m}^2/\text{s}$



(d) 600 $\mu\text{molPhoton/m}^2/\text{s}$



(e) 60 $\mu\text{molPhoton/m}^2/\text{s}$ with a younger (6-day-old) inoculum

Fig. 2. Viability measurement of the course of the trophic conversion experiments. Viability obtained by Propidium iodide (yellow green laser 610/20 nm detection)/Fluoresceine DiAcetate (blue laser, 530/30 nm detection) dual staining, optimized for *Chlorella vulgaris* (2). 100 000 events recorded per test. Biological triplicate. Points - Measurements.

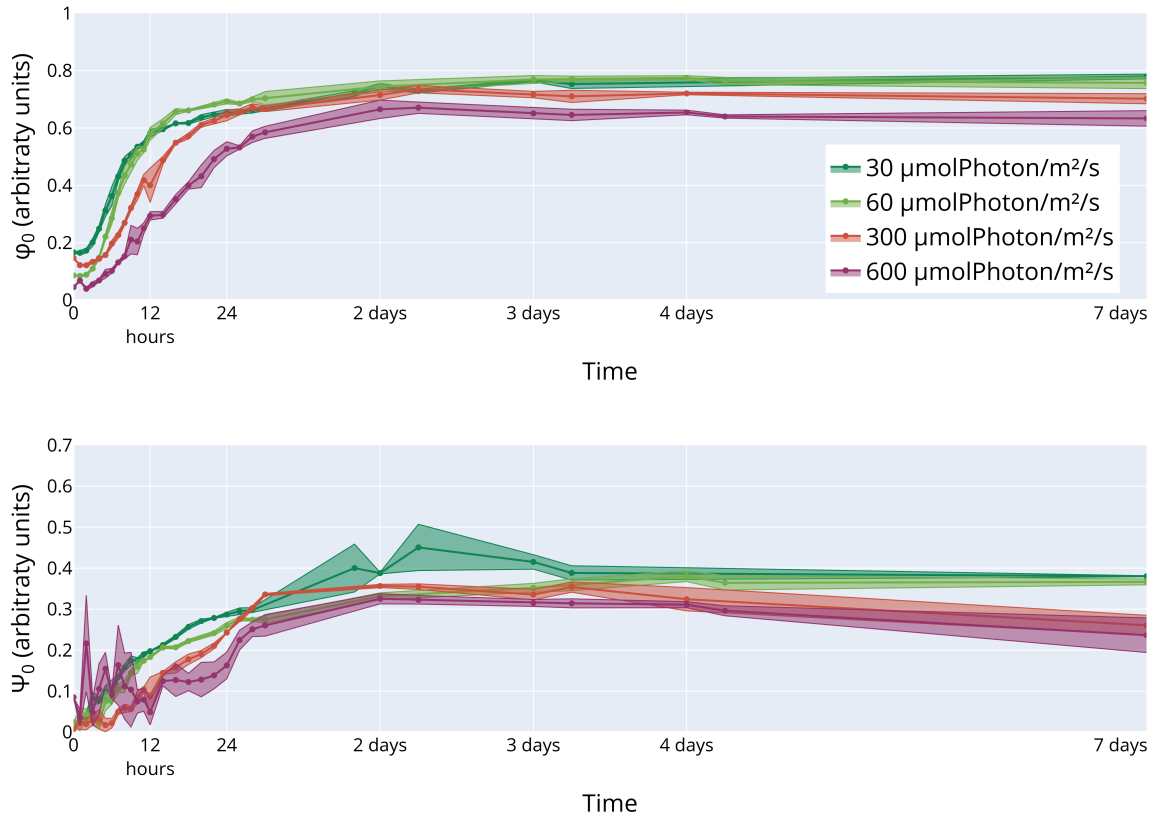


Fig. 3. Evolution over time of the indicators tied to the PSII status. ϕ_0 (Top) and Ψ_0 (Bottom) obtained via fast fluorescence induction assays (a.k.a. OJIP tests). Solid line - mean of the three replicates. Shaded area - standard deviation ($n=3$). Points - Measurements. For the run under 300 $\mu\text{molPhoton/m}^2/\text{s}$, the measurement at time 24 h for the third replicate yielded an artifact (5 times higher values than the two others and 5 times higher value than the previous and the next measurement). The value was therefore replaced by the average of the two surrounding values.

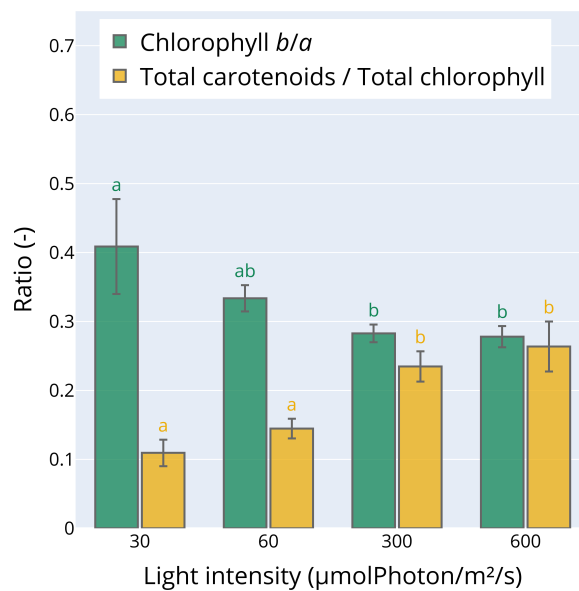


Fig. 4. Pigment ratio obtained from end-point analysis. Presented as average and standard deviation ($n=3$). Letters - Statistical significance (ANOVA, post-hoc Honestly Significant Difference with Bonferroni correction, $p < 0.05$).

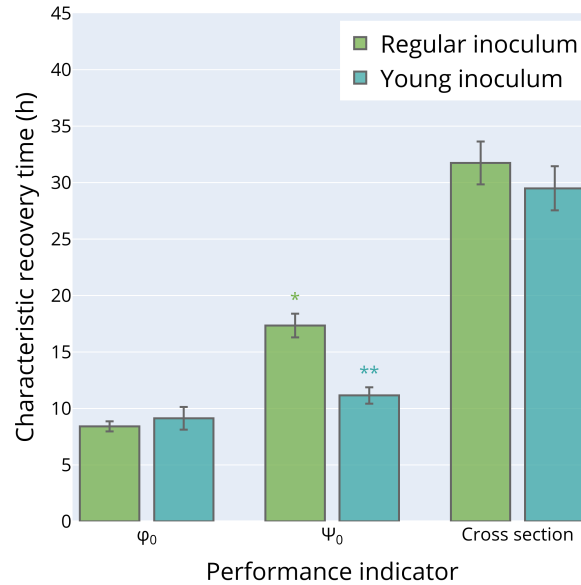


Fig. 5. Characteristic times of PSII indicators recovery and pigment expression. Regular Pigment ratio obtained from end-point analysis. Regular inoculum has a preparation duration of 11 days. Younger inoculum has a preparation duration of 6 days. All preparation conditions were kept the same between the two tests. The trophic conversion process was led under 60 $\mu\text{molPhoton}/\text{m}^2/\text{s}$. Presented as average and standard deviation ($n=3$). Stars - Statistical significance (Welch's t-test, two-sided, star $p < 0.05$).

Component	Concentration in B3N (mM)	Concentration used in 2xB3N (mM)
Glucose	0	111
NaNO ₃	8.82	17.64
CaCl ₂ · 2H ₂ O	1.70×10^{-1}	3.40×10^{-1}
MgSO ₄ · 7H ₂ O	3.04×10^{-1}	6.08×10^{-1}
K ₂ HPO ₄	4.31×10^{-1}	8.62×10^{-1}
KH ₂ PO ₄	1.29	2.58
NaCl	4.28×10^{-1}	8.56×10^{-1}
EDTA	1.71×10^{-1}	3.42×10^{-1}
KOH	5.53×10^{-1}	11.06×10^{-1}
FeSO ₄ · 7H ₂ O	1.79×10^{-2}	3.58×10^{-2}
H ₂ SO ₄	1.87×10^{-2}	3.74×10^{-2}
H ₃ BO ₃	1.35×10^{-1}	2.70×10^{-1}
ZnSO ₄ · 7H ₂ O	3.07×10^{-2}	6.14×10^{-2}
MnCl ₂ · 4H ₂ O	7.28×10^{-3}	14.56×10^{-3}
MoO ₃	4.93×10^{-3}	9.86×10^{-3}
CuSO ₄ · 5H ₂ O	6.29×10^{-3}	12.58×10^{-3}
Co(NO ₃) ₂ · 6H ₂ O	1.68×10^{-3}	3.36×10^{-3}

Table 1. Composition of the culture media used in this study. They are based on B3N medium described by Andersen (3). Stock solutions were conditioned following the guidelines mentioned hereinbefore. Carbon sources and nitrogen sources were autoclave sterilized separately before being mixed together under sterile conditions and manipulations. Formulated media were used within 7 days after preparation and stored at room temperature (to avoid precipitation) in the meantime.

References

1. Reto Jörg Strasser, Alaka Srivastava, and M. Tsimilli-Michael. The fluorescence transient as a tool to characterize and screen photosynthetic samples. *Probing photosynthesis: mechanisms, regulation and adaptation*, pages 445–483, 2000.
2. Victor Pozzobon, Wendie Levasseur, Elise Viau, Emilie Michiels, Tiphaine Clément, and Patrick Perré. Machine learning processing of microalgae flow cytometry readings: illustrated with *Chlorella vulgaris* viability assays. *Journal of Applied Phycology*, 32(5):2967–2976, October 2020. ISSN 1573-5176.
3. Robert A. Andersen and Phycological Society of America. *Algal Culturing Techniques*. Academic Press, February 2005. ISBN 978-0-12-088426-1. Google-Books-ID: 9NADUHyFZaEC.