Supplementary information for Manuscript

Title: High-throughput optimisation of light-driven microalgae biotechnologies
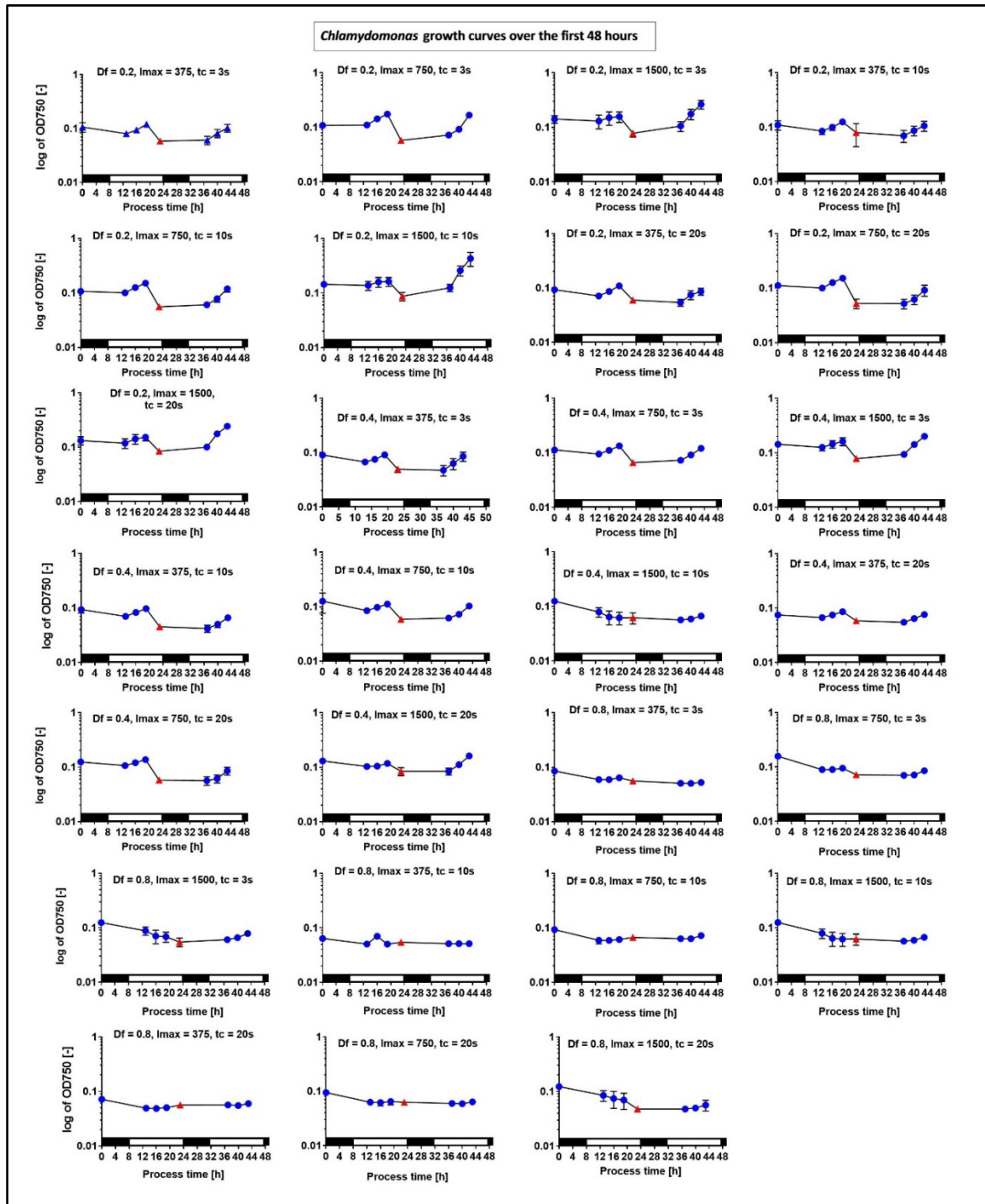
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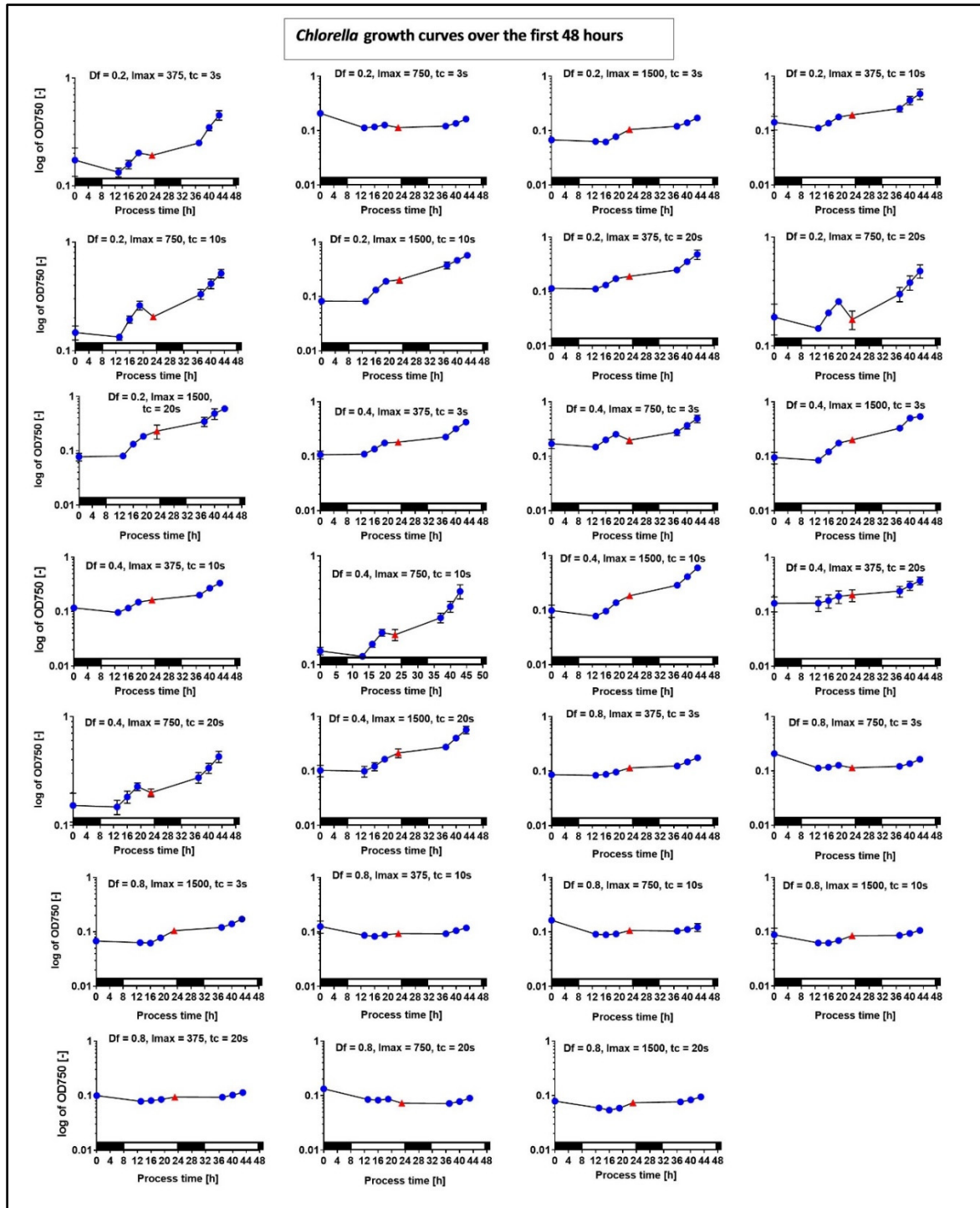
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Supplementary Figure S1. Growth curves of *Chlamydomonas* over the first 48 hours for the 27 conditions tested. Red triangles represent the OD measurement after dilution. Bottom bars represent the duration of the 16/8 h light/dark cycles: black = dark period and white = light period with the respective fluctuating light regime. The points above the first white bar represent Day 1 (acclimation phase) and the points after the red triangle represent Day 2 (quasi-steady state) data used for growth rate and PE_μ calculations. All data are mean of 3 replicates ± standard deviation.

Supplementary Figure S2. Growth curves of *Chlorella* over the first 48 hours for the 27 conditions tested. Red triangles represent the OD measurement after dilution. Bottom bars represent the duration of the 16/8 h light/dark cycles: black = dark period and white = light period with the respective fluctuating light regime. The points above the first white bar represent Day 1 (acclimation phase) and the points after the red triangle represent Day 2 (quasi-steady state) data used for growth rate and PE_{μ} calculations. All data are mean of 3 replicates ± standard deviation.
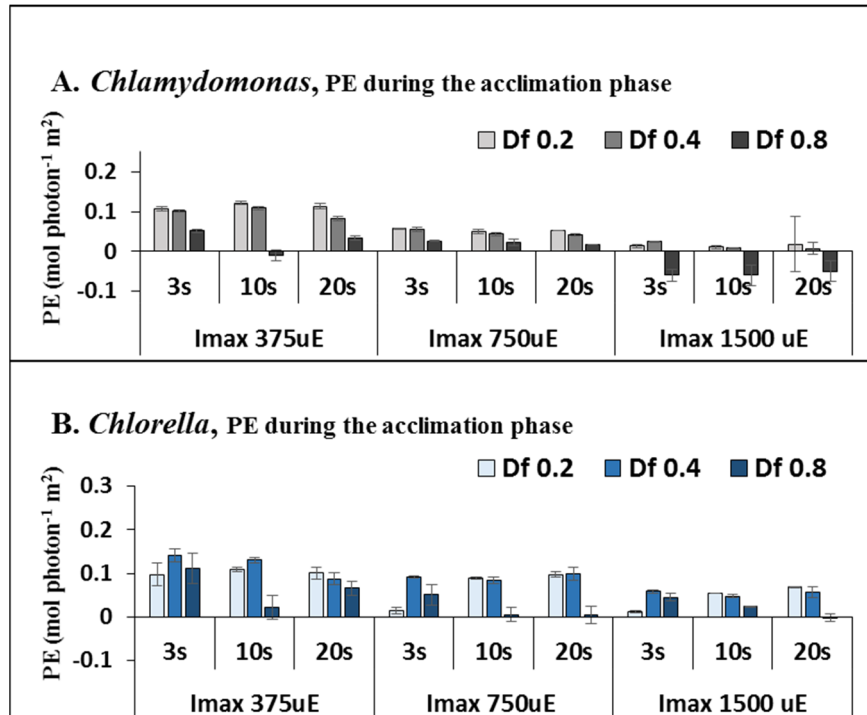
Supplementary Figure S3. Trends in photosynthetic efficiency (PE_μ, mol photon^{-1} m^2) under the 27 different light regimes for Chlamydomonas (grey bars) and Chlorella (blue bars) during the acclimation phase (Day1, A and B).

Supplementary Table S1. PE_μ of *Chlamydomonas* and *Chlorella* under constant light of the same average irradiance as fluctuating light treatments of each unique combination of I_{max} and D_f (as per Table 1 in the main text). All data represent the mean of 3 replicates ± standard deviation
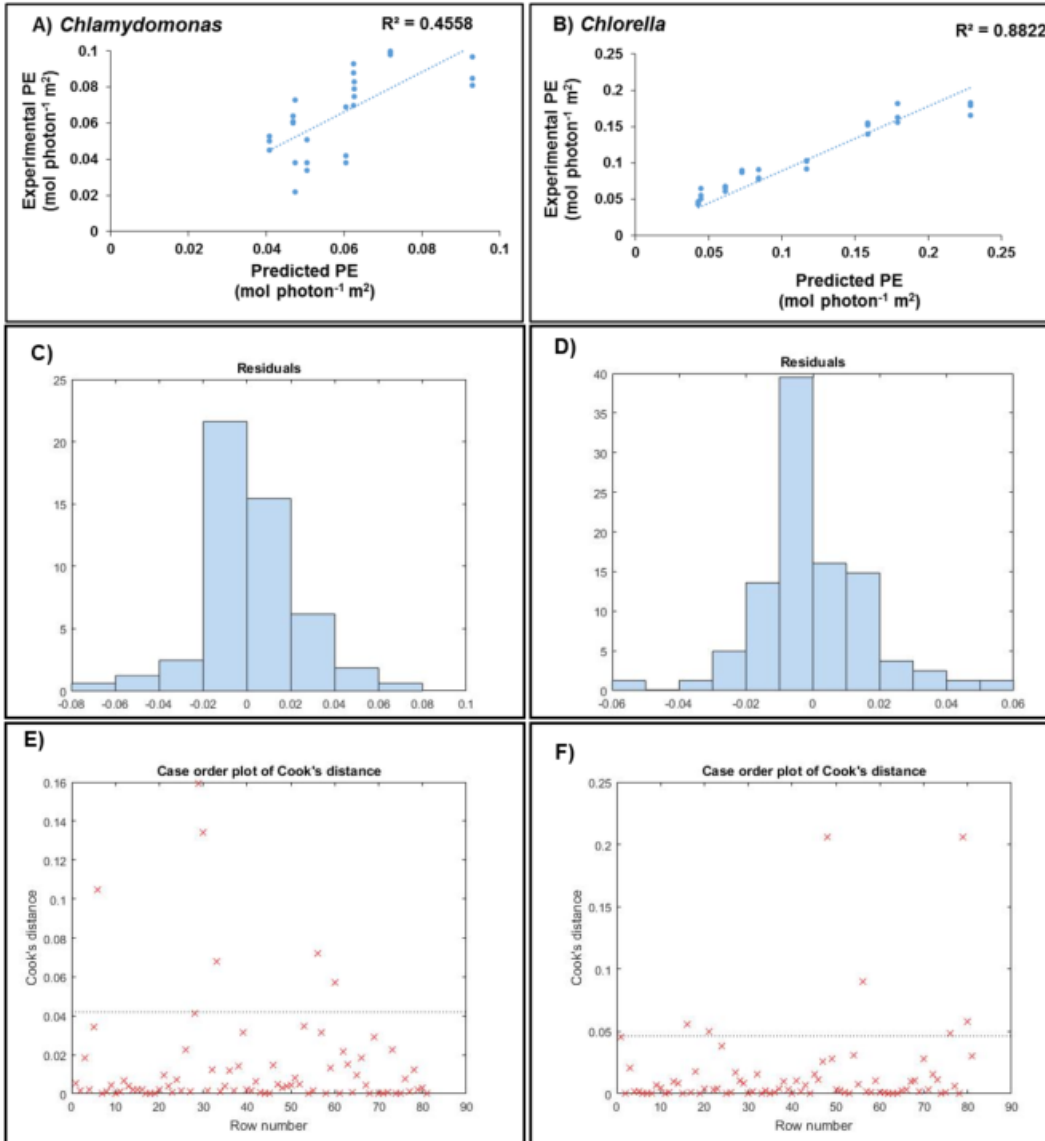
I_{avg}	PE_μ under <u>constant light</u> (mol photon^{-1} m^2)					
(mol m^{-2} h^{-1})	*Chlamydomonas*			*Chlorella*		
0.619	0.149	±	0.004	0.120	±	0.019
0.490	0.169	±	0.008	0.151	±	0.006
0.367	0.175	±	0.008	0.178	±	0.010
0.180	0.178	±	0.040	0.253	±	0.026
1.242	0.054	±	0.004	0.103	±	0.003
0.979	0.113	±	0.000	0.058	±	0.007
0.738	0.116	±	0.013	0.078	±	0.002
0.360	0.197	±	0.006	0.050	±	0.002
2.480	0.045	±	0.010	0.011	±	0.000
1.958	0.061	±	0.004	0.029	±	0.001
1.472	0.055	±	0.004	0.051	±	0.001
0.713	0.111	±	0.010	0.049	±	0.005

PE$_\mu$ under constant light (control)
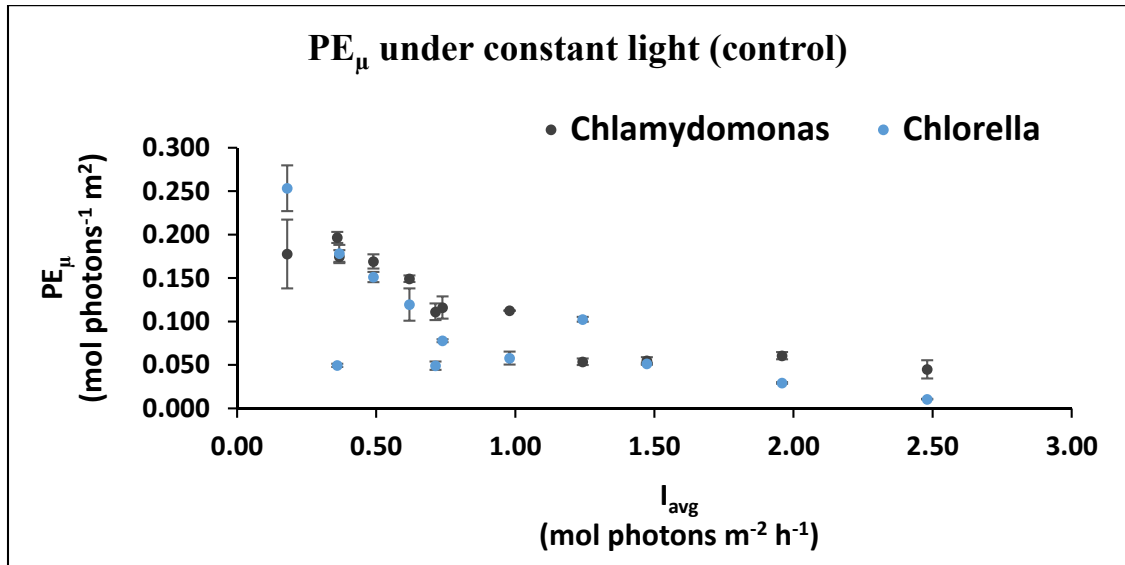
Supplementary Figure S4. Trends in photosynthetic efficiency (PE$_\mu$, mol photon^{-1} m^2) under constant light for *Chlamydomonas* and *Chlorella* of the same I$_{avg}$ as fluctuating light regimes of each unique combinations of I$_{max}$ and D$_f$ on Day 2. Error bars represent standard deviations (n=3).

Supplementary Figure S5. Model validation using an independent data set. **A** and **B** display predicted versus experimental PE_μ data (to confirm sufficient goodness of fit), **C** and **D** display residuals plot (to confirm normal distribution) and **E** and **F** Cook's distance plot (to identify outliers) for *Chlamydomonas* and *Chlorella* respectively.

Supplementary Table S2. Factor coefficients obtained from analysis of variance (ANOVA) for NPQ and OD_{680}/OD_{750}. All data represent the mean of 3 replicates ± standard deviation. [*] represents significant effects at p-value<0.05
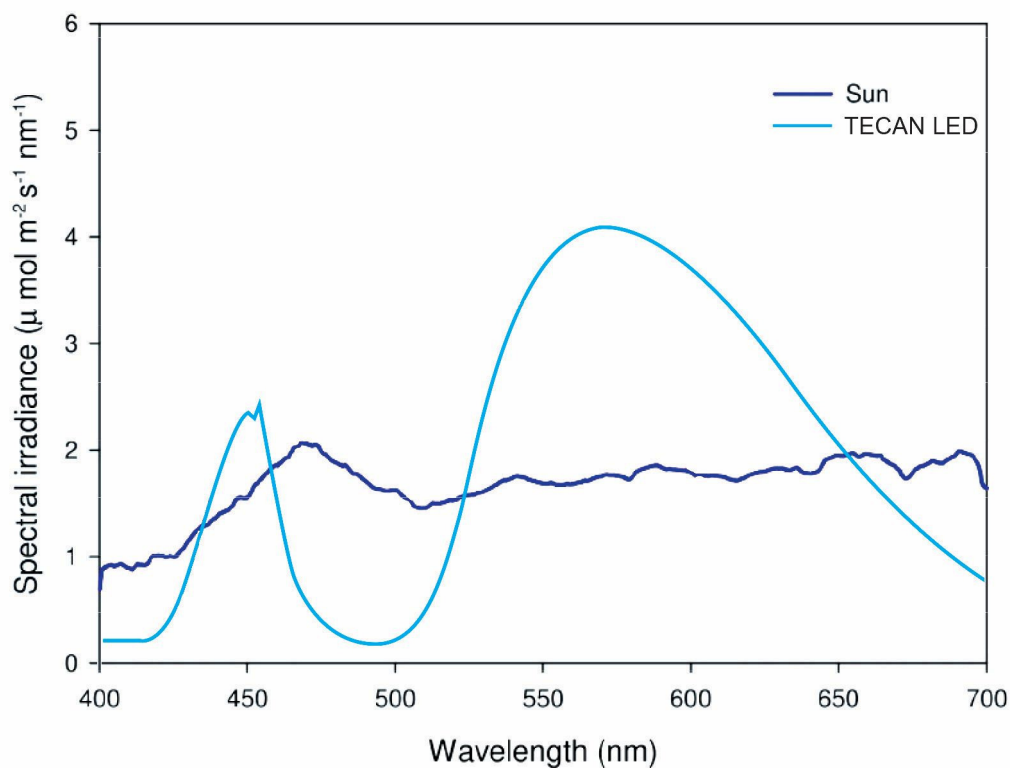
	Coefficients from the quadratic non-linear model			
	NPQ		OD_{680}/OD_{750}	
	Chlamydomonas	*Chlorella*	*Chlamydomonas*	*Chlorella*
D_f	-0.0026	-0.0155[*]	0.2514[*]	0.1879[*]
I_{max}	0.0267[*]	-0.0134[*]	-0.2208[*]	0.0300[*]
t_c	0.0061	-0.0046	0.0106	-0.0674[*]
D_f - I_{max}	0.0006	-0.0098	0.0408[*]	0.02153
D_f - t_c	0.0056	-0.0008	-0.0162	0.0705[*]
I_{max} - t_c	0.0058	0.0003	0.0163	-0.0471[*]
D_f^2	-0.0313[*]	-0.0026	0.1206[*]	0.3359[*]
I_{max}^2	0.0141	-0.0101	0.0619[*]	-0.0143
t_c^2	0.0081	-0.0056	0.0078	-0.0093
Intercept	0.1729	0.0933	2.0436	1.734
R^2	0.49	0.37	0.89	0.85

Supplementary Table S3. Cell synchronisation procedure. The acclimation procedure for the light regime experiments based on the I_{max} considered. "Ticks" or "x" represent that particular step was or was not performed for the corresponding I_{max} experiment respectively. Note: For the higher intensity (I_{max} 1500), care was taken not to shock the cultures by subjecting them to a step-wise gradually increasing light regime.

Duration	Samples	Light regime	I_{max} 375	I_{max} 750	I_{max} 1500
~ 2-3 days	**Pre flasks** on shakers outside the TECAN	Constant overhead light ~100 µE	☑	☑	☑
~ 2-4 days	**Pre-flasks** in TECAN with overhead lights	Constant ~100 µE L/D 16/8 h	☑	☒	☒
~ 2 days	**Pre-flask**s in TECAN with LED's	TECAN LED's 100µE constant light; L/D 16/8 h	☑	☒	☒
~ 24 hours	**Pre-flasks** in TECAN with LEDs	Constant 350 µE LEDs , L/D 16/8 h	☒	☒	☑
Pre flasks, 2 hours	**Pre-flasks** in TECAN	400 µE (1 h), 600 µE (1 h)	☒	☒	☑
1hour	Cultures adjusted to target starting OD$_{750}$; Plated in **96 well plates**	TECAN LED's at100µE constant light; L/D 16/8 h	☑	☑	☒
Start of experiment	96-well plates-light regime is set.	Considered ready for measurements as mentioned in the Methods section. L/D 16/8 h	☑	☑	☒
Day 0, after plating	96 well plates- gradual light acclimation in progress for higher I_{max} experiment.	1 h 200 µE constant 2 h light cycles with I_{max} = 750 µE First measurement as mentioned in the Methods section 8h dark period	☒	☒	☑
Day 1, AM, gradual acclimation	Acclimation of plates continue- before making the first dilution	First 4 h of light after dark period: Light regime with I_{max} = 750 µE Next 6h – light regime with I_{max} = 1500 µE followed by dilution of samples	☒	☒	☑
Day 1, afternoon	Acclimation of plates continue- Post-dilution of plates. Acclimation set.	2.5 h light regimes with I_{max} = 750 µE (for recovery after dilution) 2 h light regime with I_{max} = 1000 µE 1.5 h light regime with I_{max} = 1500 µE 8 h dark period	☒	☒	☑
Day 2, AM	Acclimation continues	10 h light regime with I_{max} = 1500 µE followed by dilution of cultures.	☒	☒	☑
Day 2, afternoon onwards up to Day 3 afternoon	Acclimation continues	Repeat same as Day 1 afternoon onwards	☒	☒	☑

Supplementary Figure S6. Approximation of the spectral irradiance over the PAR spectrum (400-700nm) showing the difference between sunlight (dark blue line) and the warm white LEDs of the TECAN system (light blue line). Sun spectrum reproduced from Yarnold, J.[1]. TECAN spectrum reproduced from World of Thought Pty Ltd, Australia (www.worldofthought.com.au).

Reference

1   Yarnold, J. *Photosynthesis of microalgae in outdoor mass cultures and modelling its effects on biomass productivity for fuels, feeds and chemicals* PhD thesis, The University of Queensland, (2016).