

A new approach for sustained and efficient H₂ photoproduction by *Chlamydomonas reinhardtii*

Sergey Kosourov*, Martina Jokel, Eva-Mari Aro and Yagut Allahverdiyeva*

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*Molecular Plant Biology, Department of Biochemistry,
University of Turku, Turku, FI-20014, Finland.*

**Corresponding authors:*

*Dr. Sergey Kosourov, e-mail: serkos@utu.fi, tel:
+358451577800;*

*Dr. Yagut Allahverdiyeva, e-mail: allahve@utu.fi, tel:
+358503506181.*

Figure S1. Photochemical activity in the wild-type *Chlamydomonas reinhardtii* culture exposed to a train of 1 s light pulses interrupted by 9 s dark periods. Before measurements, cell suspensions at around $14 \mu\text{g mL}^{-1}$ Chl (*a + b*) were dark adapted for 10 min in the gas tight cuvette under either Ar or air atmosphere. A train of saturating white light pulses ($\sim 500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was provided with the pulse amplitude modulated fluorometer (Dual-PAM-100, Walz). Arrows indicate the points for switching on / off the measuring light. Algal suspension was stirred throughout the experiment. In the case of anaerobic incubation, the presence of H_2 gas in the headspace of the cuvette was confirmed with a GC.

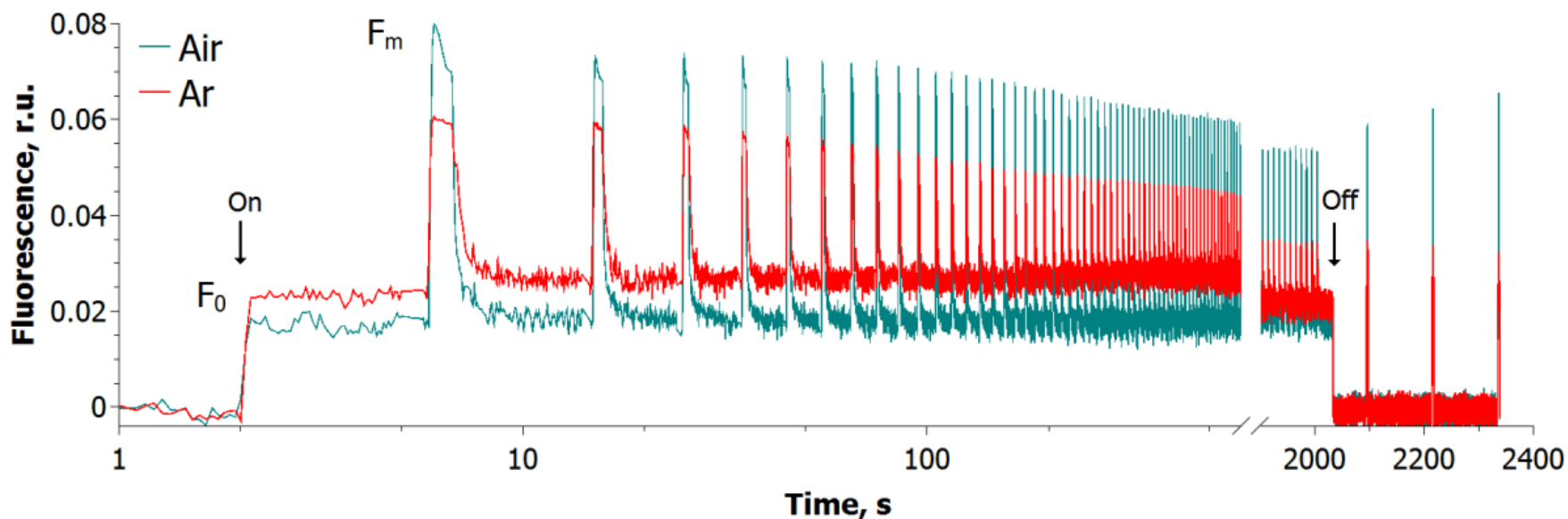


Figure S2. H₂ photoproduction and O₂ evolution in algal cultures exposed to continuous light. The cultures were treated with different amounts of 1 s light pulses (420 μmol photons m⁻² s⁻¹) interrupted by 9 s dark periods (shown as a number of cycles), dark-adapted for 2 minutes, and then continuous light of the same intensity was applied. The arrows indicate the points of continuous light application.

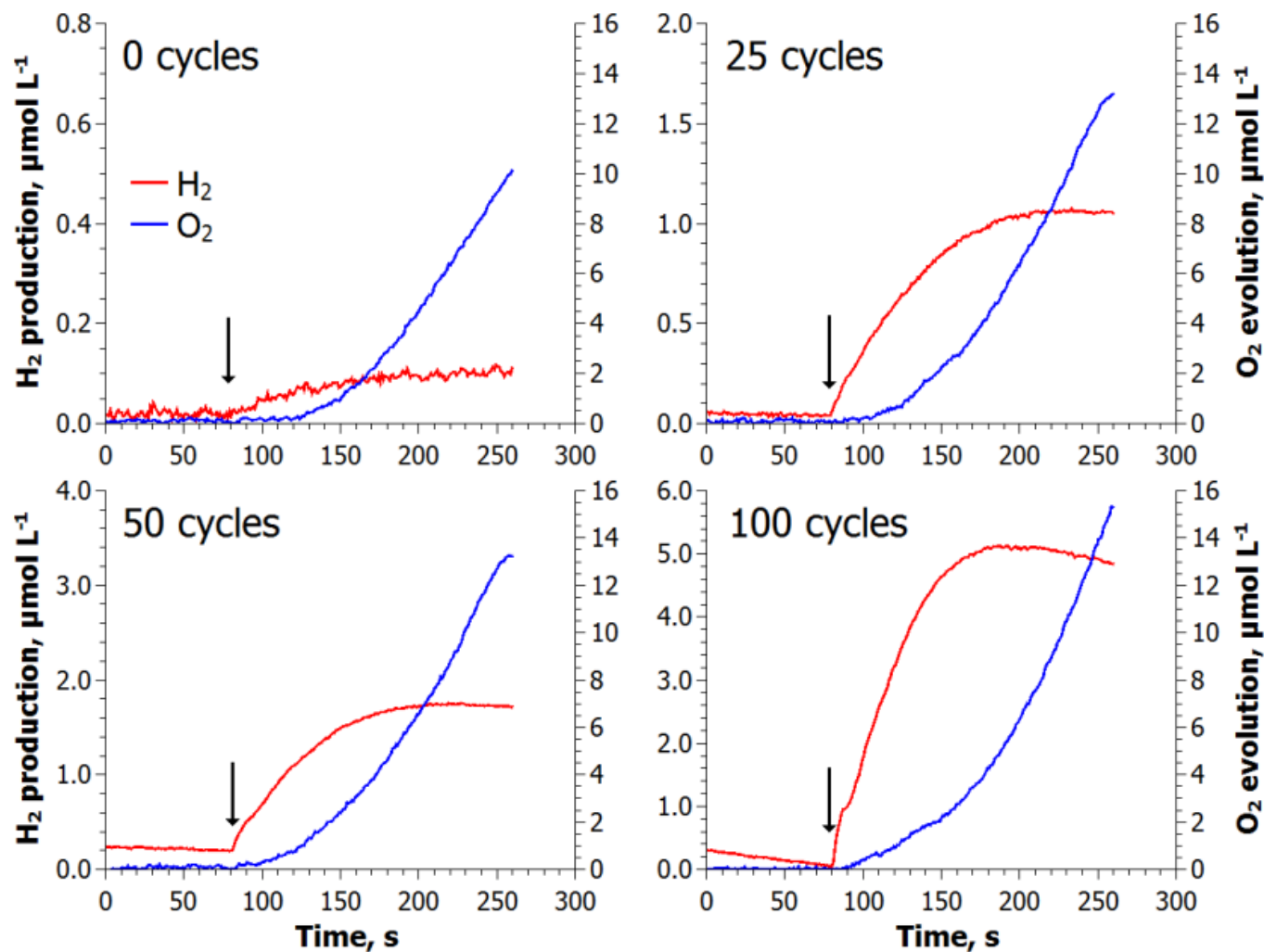


Figure S3. Mass spectrometric simultaneous monitoring of H₂, O₂ and CO₂ exchange in algal cultures exposed to a train of 1 s light pulses (420 μmol photons m⁻² s⁻¹) interrupted by 3 s dark periods.

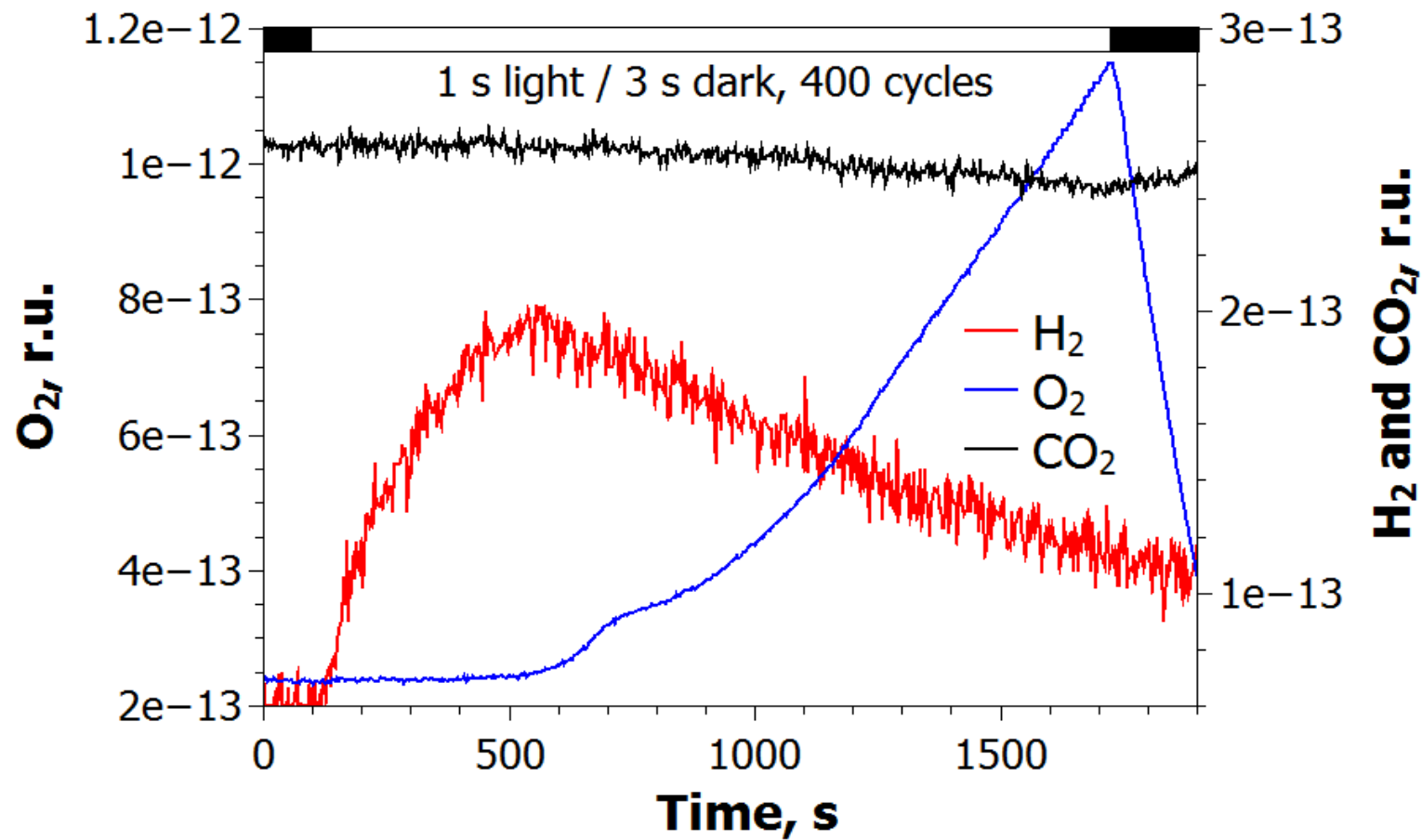
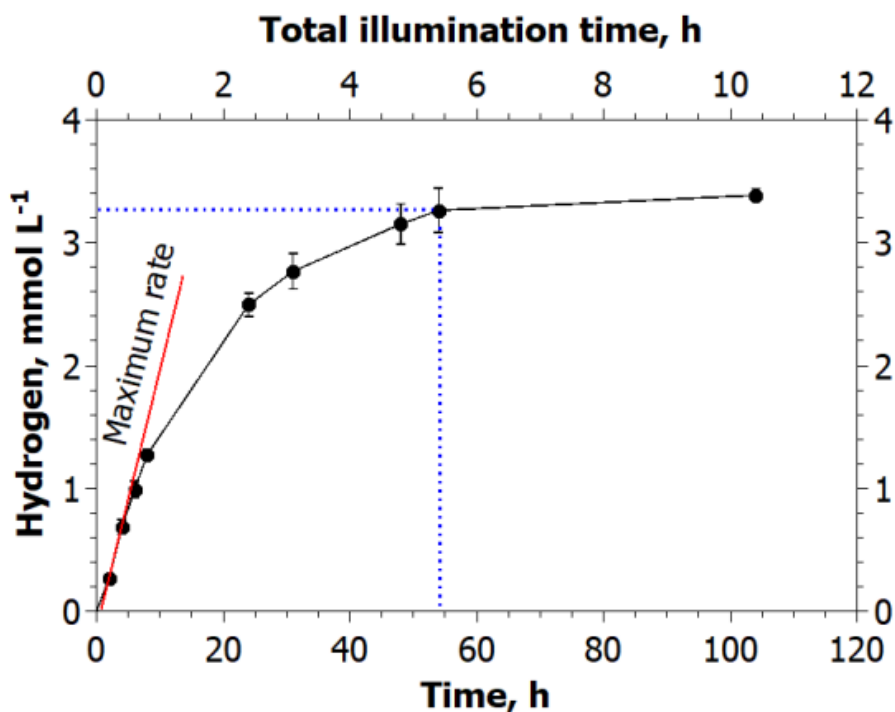


Figure S4. Evaluation of light energy to H₂ energy conversion efficiency (LHCE) of the pulse-illuminated *C. reinhardtii* cultures. 10 ml algal suspensions with the total Chl content of about 18 mg L⁻¹ were exposed to a train of 1 s light pulses (35.11 W m⁻² PAR) interrupted by 9 s dark periods under Ar atmosphere. The LHCEs were calculated for the maximum rate of H₂ photoproduction (red line) and for the 54 h production period using eqn (1). 35.11 W m⁻² corresponds to 180 μmol photons m⁻² s⁻¹ white light at the surface of the suspension culture.

$$\eta (\%) = 100 \frac{\left(\Delta G^{\circ} - RT \ln \left(\frac{P^{\circ}}{P} \right) \right) V_H}{E_S t A} \quad (1)$$

where ΔG° is the change of the standard Gibb's free energy for the water-splitting reaction (237,200 J mol⁻¹ at 25°C and 1 atm), R is the universal gas constant, T is the absolute temperature, P° and P are the standard and observed H₂ pressures (atm), V_H is the amount of H₂ photoproduced (mol), E_S is the energy of the incident light radiation (J m⁻² s⁻¹), A is the illuminated surface area (m²) and t is a sum of illumination periods (s).

(A) Original H₂ photoproduction data



(B) Parameters and final efficiencies

	Max. rate	H ₂ yield (54 h)
V_H (mol):	4.20134E-6	3.26347E-5
P (atm):	0.0028	0.0133
A (m ²):	0.002178	0.002178
E_S (J m ⁻² s ⁻¹):	35.11	35.11
t (s):	720	19440
η (%):	1.7	0.5
η_{upper} (%):	2.2	0.63

* η_{upper} calculated for the upper H₂ gas combustion energy (ΔH_c) of 285.8 kJ mol⁻¹.