Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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Photocatalytic Hydrogen Evolution with a Hydrogenase in a Mediator-Free System under High Levels of Oxygen**

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Contents

Experimental Section.

Materials and methods. Eosin Y (Na_2EY), $[Ru(2,2'\text{-bipyridine})_3]Cl_2$, MV and TEOA (titrated with dilute NaOH to the desired pH) were purchased from commercial suppliers in the highest available purity.

Isolation and purification of *Db* [NiFeSe]-H_2ase. The [NiFeSe]-hydrogenase from *Desulfomicrobium baculatum* (*Db* [NiFeSe]-H_2ase), which was purified using a previously published method[1] was a kind gift from Dr. Juan C. Fontecilla-Camps *et al.*

Photocatalytic H_2 evolution experiments. The homogeneous H_2 evolution system consisting of *Db* [NiFeSe]-H_2ase and EY was prepared by the following method: Na_2EY was added to an aqueous TEOA solution (2.25 mL, 150 mM, pH 7.0) in a photoreactor (2.26 mL or 5.46 mL headspace volume) followed by the addition of *Db* [NiFeSe]-H_2ase in an anaerobic MBraun glovebox. The photoreactor was sealed with a rubber septum and taken out of the glovebox. Subsequently, the photoreactor was purged through the solution with 2% CH_4 in N_2 for 15 min, which was used for all experiments. The sealed photoreactor was thermostated with a water jacket at 25 °C. For photocatalytic experiments, white light from a solar light simulator (Newport Oriel, 100 mW cm^{-2}, AM 1.5G) and single wavelength LED light source (525nm, controlled by CompactStat, IVIUM) were employed. Infrared (IR) irradiation was filtered with a water filter and UV irradiation with a 420 nm longpass filter (UQG Optics, FGG-42050) in all experiments where the solar light simulator was used. Short photoreactors (2.26 mL headspace volume) were used for optimization of the system (Figure S1-S4) and air exposure experiments (Figure S5). Long photoreactors (5.46 mL headspace volume) were used for O_2 and CO exposure experiments. O_2 and CO atmospheres were created by injecting pure O_2 or CO into the photoreactor.

Headspace H_2 and O_2 measurements. The amount of gaseous H_2 and O_2 was detected and quantified by headspace gas analysis using an Agilent 7890A Series GC equipped with a 5 Å molecular sieve column. The GC oven holding the columns was kept at 45 ºC. Aliquots of at least 20 μL of the headspace gas were removed for GC analysis. CH_4 (2%) in N_2 was used as internal standard in all experiments.

Measurement of O_2 concentration in solution. The concentration of O_2 was determined by a fluorescence-based O_2 probe (Neofox - Ocean Optics), which was inserted into the solution and covered from direct light irradiation. Concentration in ppm was measured by the provided software, after a built-in calibration was performed. The background signal under 0% O_2 was subtracted from all measurements.

Calculation of the External Quantum Efficiency (EQE)

Hydrogen generation n_{H2} (mol H_2) was driven by green light ($\lambda = 525$ nm) of intensity I at 25 ºC. EQE can be calculated with the following formula:

$EQE = (2 \cdot n_{H2} \cdot N_A \cdot h \cdot c)/(t_{irr} \cdot \lambda \cdot I \cdot A) \cdot 100\%$

Where N_A is the Avagadro constant, h is the Planck constant, c is speed of light, t_{irr} is the irradiation time, A is the irradiated area of the photoreactor.

Treatment of data.

All experiments were conducted at least three times. Unweighted mean value (x_u) and the standard deviation (σ) were calculated the following way:

$x_u = \Sigma_i x_i/n$; $\sigma = \{\Sigma_i (x_i - x_u)^2 / [n(n-1)]\}^{1/2}$

Where (n) is the number of times the experiment was run, (x_i) the observation at a particular experiment. Standard deviation of values, which have been calculated from values with errors, was determined using the error propagation. TOFs were calculated based on the first hour of irradiation in the Supporting Information unless stated otherwise.

Reference

[1] Hatchikian, E. C.; Bruschi, M.; Le Gall, J.; Forget, N.; Bovier-Lapierre, G. *Biochem. Biophys. Res. Commun.* 1978, *82*, 451–461.

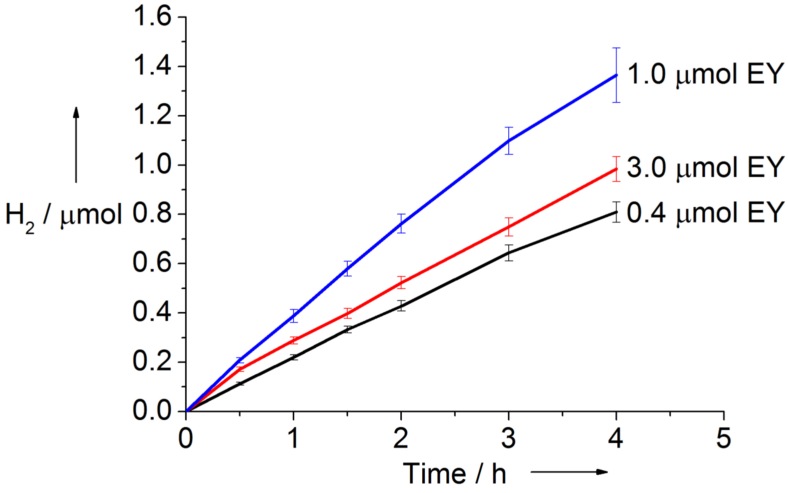


Figure S1. Visible light-driven (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of *Db* [NiFeSe]–H_2ase (10 pmol) in an aqueous TEOA solution (150 mM) at pH 7.0 and 25 °C, in the presence of 1.0 μmol, 3.0 μmol and 0.4 μmol of EY. Standard deviations are given as vertical error bars.

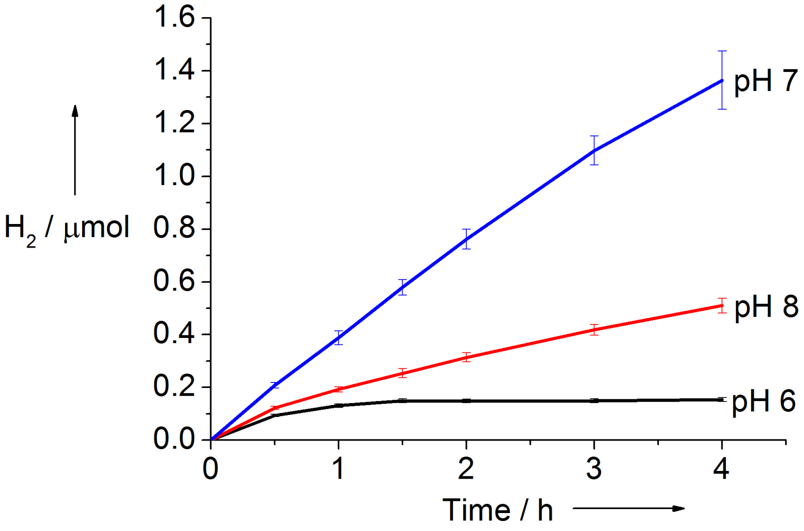


Figure S2. Visible light-driven (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of *Db* [NiFeSe]–H_2ase (10 pmol) and EY (1.0 μmol) in an aqueous TEOA solution (150 mM) at 25 °C at pH 7.0, pH 8.0 and pH 6.0. Standard deviations are given as vertical error bars.

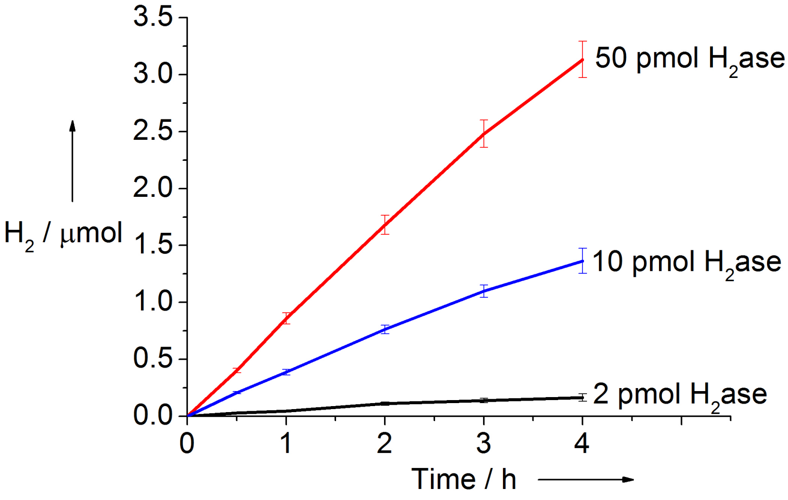


Figure S3. Visible light-driven (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of EY (1.0 μmol) in an aqueous TEOA solution (150 mM) at pH 7.0 and 25 °C, in the presence of 50 pmol, 10 pmol and 2 pmol of *Db* [NiFeSe]–H_2ase. Standard deviations are given as vertical error bars.

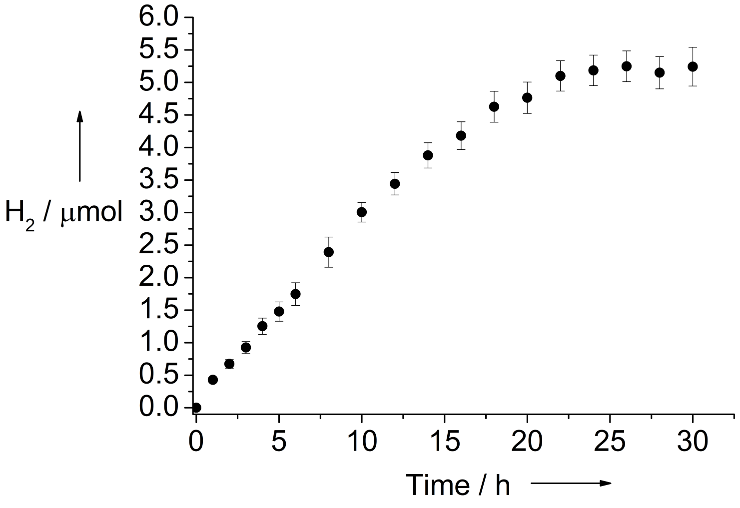


Figure S4. Long-term visible light-driven (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of *Db* [NiFeSe]–H_2ase (10 pmol) and EY (1.0 μmol) in an aqueous TEOA solution (150 mM) at pH 7.0 and 25 °C. Standard deviations are given as vertical error bars.

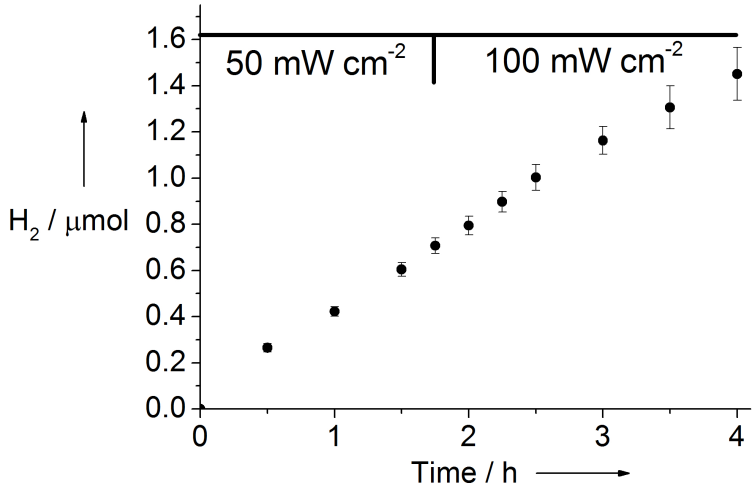


Figure S5. Visible light-driven (AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of *Db* [NiFeSe]–H_2ase (10 pmol) and EY (1.0 μmol) in an aqueous TEOA solution (150 mM) at pH 7.0 and 25 °C at 50 and 100 mW cm^{-2}. Standard deviations are given as vertical error bars.

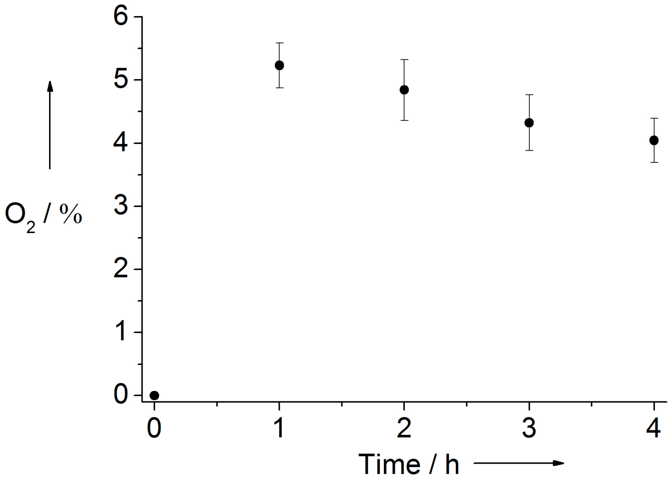
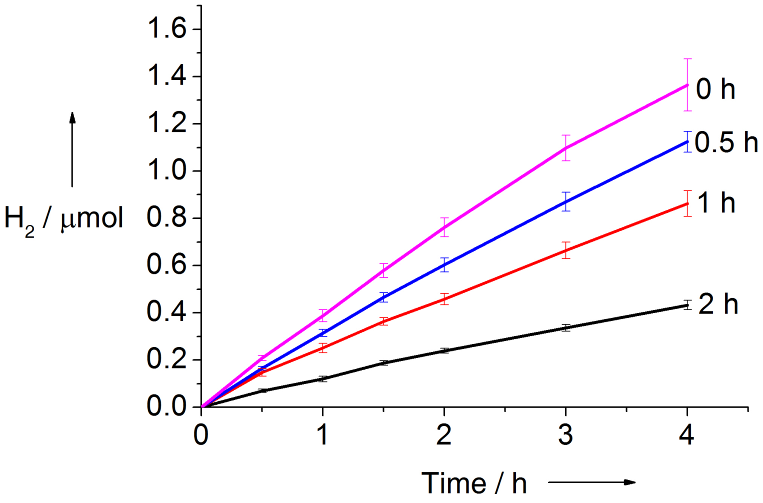


Figure S6. O_2 levels measured by GC during visible light irradiation (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) of *Db* [NiFeSe]–H_2ase (10 pmol) and EY (1.0 μmol) in an aqueous TEOA solution (150 mM) at pH 7.0 and 25 °C, where O_2 was injected into the headspace after one hour of irradiation resulting in 5% of O_2 in the headspace. Standard deviations are given as vertical error bars.



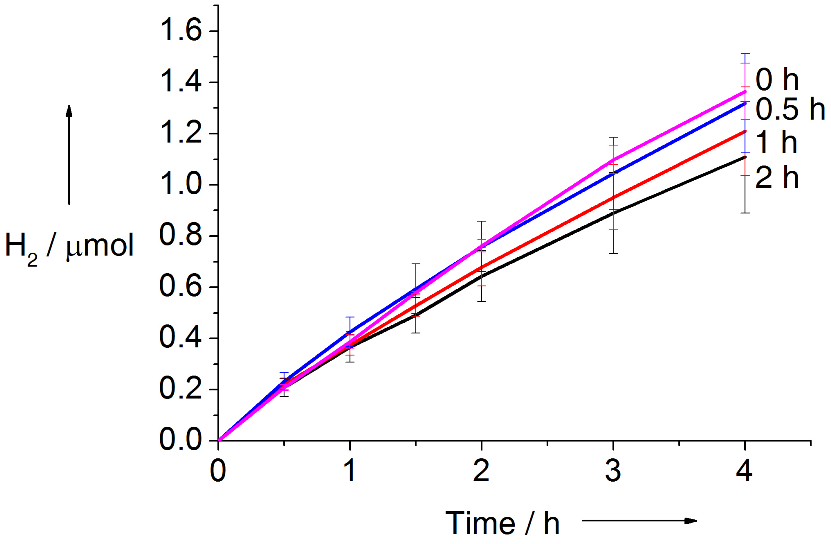


Figure S7. Visible light-driven (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of *Db* [NiFeSe]–H_2ase (10 pmol) and EY (1.0 μmol) in an aqueous TEOA solution (150 mM) at pH 7.0 and 25 °C after exposure to air under (top) continuous visible light irradiation and (bottom) dark conditions for 0 min, 30 min, 60 min and 120 min. Standard deviations are given as vertical error bars. See also Table S3.

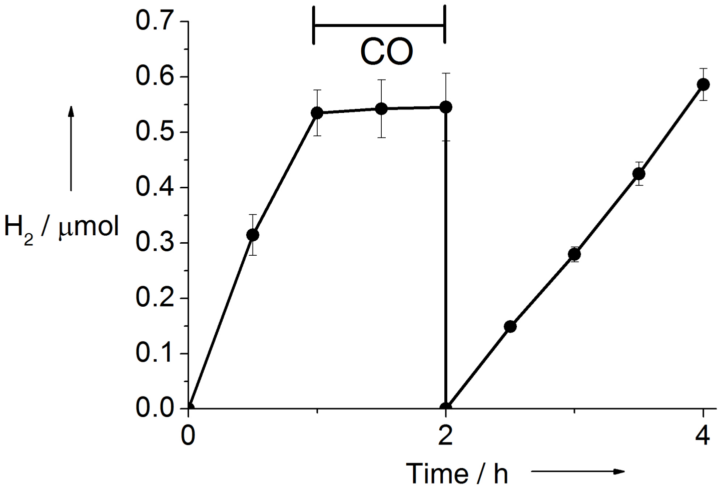


Figure S8. Visible light-driven (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of *Db* [NiFeSe]–H_2ase (10 pmol) and EY (1.0 μmol) in an aqueous TEOA solution (150 mM) at pH 7.0 and 25 °C. After the first hour of the experiment, CO was injected into the photoreactor resulting in 2% of CO in the headspace and re-purged again one hour later. Standard deviations are given as vertical error bars.

Table S1. Visible light-driven (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) H_2 production in the presence of varying amounts of EY, *Db* [NiFeSe]–H_2ase and at various pH values in an aqueous TEOA buffer solution (2.25 mL, 150 mM) at 25 °C. Note that these experiments were performed with a photoreactor with a headspace volume of 2.26 mL and the results differ therefore slightly with the ones presented in the main text.

Conditions	$TOF_{H2ase} \pm \sigma$ / s^{-1}	$H_2 \pm \sigma$ / µmol H_2 h^{-1}	H_2 (2h) $\pm \sigma^{a}$ / %
EY Dependence			
0.4 µmol	6.1 ± 0.3	0.22 ± 0.05	0.46 ± 0.02
1 µmol	10.8 ± 0.7	0.39 ± 0.03	0.82 ± 0.04
3 µmol	8.0 ± 0.4	0.29 ± 0.01	0.56 ± 0.03
pH Dependence			
pH 6.0	3.6 ± 0.2	0.13 ± 0.01	0.16 ± 0.01
pH 7.0	10.8 ± 0.7	0.39 ± 0.03	0.82 ± 0.04
pH 8.0	5.3 ± 0.3	0.19 ± 0.01	0.34 ± 0.02
Db [NiFeSe]-H_2ase Dependence			
2 pmol	6.4 ± 0.4	0.046 ± 0.01	0.12 ± 0.02
10 pmol	10.8 ± 0.7	0.39 ± 0.03	0.82 ± 0.04
50 pmol	4.8 ± 0.3	0.86 ± 0.05	1.79 ± 0.09

[a] H_2 accumulated after two hours of irradiation.

Table S2. The concentration of O_2 dissolved in a TEOA solution (2.25 mL, 150 mM, pH 7) in the dark and during visible light irradiation (100 mW cm^{-2}; AM 1.5G; $\lambda > 420$ nm) with different O_2 concentrations in the headspace (after equilibration time) at 25 °C.[a]

O_2 in the headspace / %	Light	H_2ase, EY / ppm	EY / ppm	H_2ase / ppm	$[Ru(bipy)_3]Cl_2$, MV, H_2ase / ppm
5	Off	1.8 ± 0.2	2.0 ± 0.3	2.4 ± 0.3	2.8 ± 0.2
	On	0.2 ± 0.1	0.03 ± 0.1	2.1 ± 0.3	0.3 ± 0.2
10	Off	3.4 ± 0.5	3.8 ± 0.6	4.2 ± 0.5	4.4 ± 0.2
	On	0.1 ± 0.1	0.04 ± 0.1	4.0 ± 0.5	0.3 ± 0.2
21	Off	6.4 ± 1.0	6.1 ± 0.7	6.3 ± 1.0	6.9 ± 0.4
	On	0.1 ± 0.1	0.04 ± 0.1	6.0 ± 0.9	0.3 ± 0.2

[a] 10 pmol of *Db* [NiFeSe]-H_2ase, 1.0 μmol of EY, 1 μmol of $[Ru(bipy)_3]Cl_2$ and 1 μmol MV were used.

Table S3. Visible light-driven (100 mW cm^{-2}; AM 1.5G; λ > 420 nm) H_2 production in the presence of *Db* [NiFeSe]–H_2ase (10 pmol) and EY (1.0 μmol) in an aqueous TEOA solution (2.25 mL, 150 mM) at pH 7.0 and 25 °C after exposure to air under various conditions for various periods of time.

Conditions (pre-treatment prior photo-H_2 generation)	TOF $_{H2ase}$ ± σ / s^{-1}	H_2 ± σ / µmol H_2 h^{-1}	H_2 (2h) ± σ^a / %
Irradiated for 0 min under air, and purged with 2% CH_4/N_2	10.8 ± 0.7	0.39 ± 0.03	0.82 ± 0.04
Irradiated for 30 min under air, and purged with 2% CH_4/N_2	8.7 ± 0.4	0.31 ± 0.01	0.65 ± 0.03
Irradiated for 60 min under air, and purged with 2% CH_4/N_2	7.0 ± 0.6	0.25 ± 0.02	0.49 ± 0.03
Irradiated for 120 min under air, and purged with 2% CH_4/N_2	3.3 ± 0.3	0.12 ± 0.01	0.26 ± 0.01
Exposed to air for 0 min in the dark, and purged with 2% CH_4/N_2	10.8 ± 0.7	0.39 ± 0.03	0.82 ± 0.04
Exposed to air for 30 min in the dark, and purged with 2% CH_4/N_2	11.8 ± 1.6	0.43 ± 0.06	0.8 ± 0.1
Exposed to air for 60 min in the dark, and purged with 2% CH_4/N_2	10.4 ± 1.1	0.37 ± 0.04	0.73 ± 0.08
Exposed to air for 120 min in the dark, and purged with 2% CH_4/N_2	10.2 ± 1.6	0.37 ± 0.06	0.7 ± 0.1

[a] H_2 accumulated in the headspace (2.26 mL) of the photoreactor after two hours of light irradiation.