In situ determination of photobioproduction of H_2 by In_2S_3-[NiFeSe] Hydrogenase from *D. vulgaris* Hildenborough using only visible light

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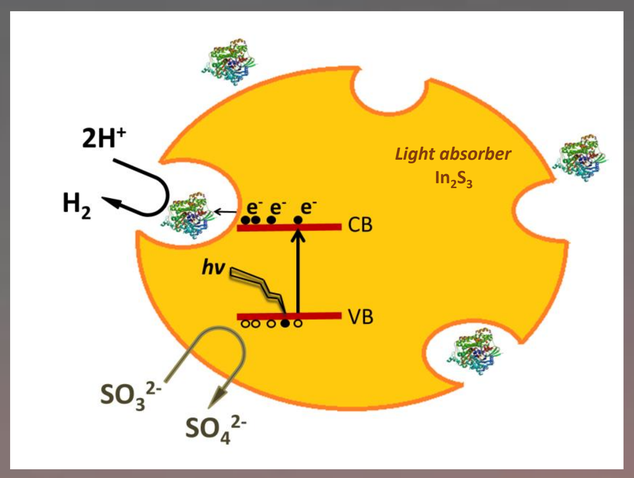


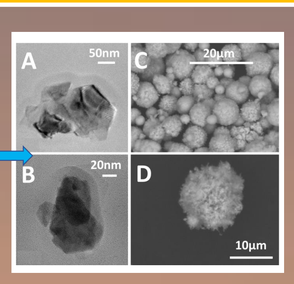
Photocatalytic hydrogen evolution is possible employing a hybrid system combining a solid-state visible light absorber, such as In_2S_3 semiconductor, with the [NiFeSe]-Hydrogenase from *Desulfovibrio vulgaris* (*Dv*-SeHase).

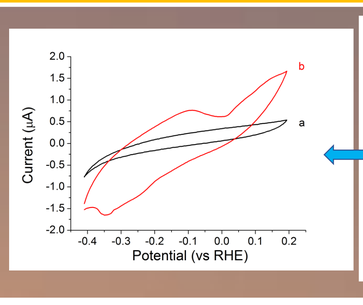
The energy of the conduction band of In_2S_3 is high enough to supply the hydrogenase with photoexcited electrons for H_2 production [1]. Direct electron transfer (DET) between the conduction band and the distal cluster of hydrogenase is aimed to obtain photocatalytic production of hydrogen.
The photocatalytic activity of soluble form of the *Dv*-SeHase incubated previously with In_2S_3 particles was measured by membrane-inlet mass spectrometry connected to an anaerobic vessel with no gas phase, under visible light irradiation [2].

The In_2S_3 light absorber characterization

In_2S_3 structure and shape

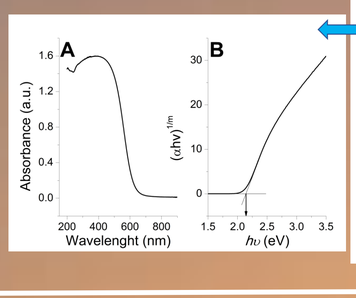
TEM images (A,B) show hexagonal nanocrystal shape particles ranging 50-100nm in diameter. SEM images (C, D) show flower like spherical particle aggregation with 2-15µm of diameter and high level of porosity.





Redox potential under irradiation

Electrochemical characterization of In_2S_3 deposited on LDG electrode. A value of standard redox potential of ≈ -0,2 vs. RHE under irradiation (b) indicates that there is a fair overpotential to make possible the electron transfer to hydrogenase.

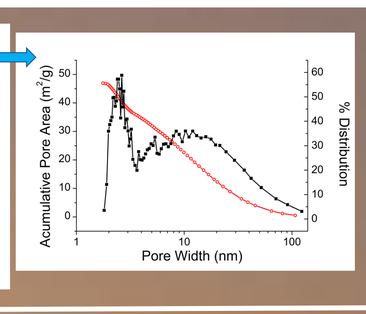


The band-gap energy

Diffuse reflectance UV-Vis spectrum (A) and band-gap determination (B) show that In_2S_3 absorbs in the range of 350-550nm with a band-gap of 2,1eV.

Pore size for hydrogenase insertion

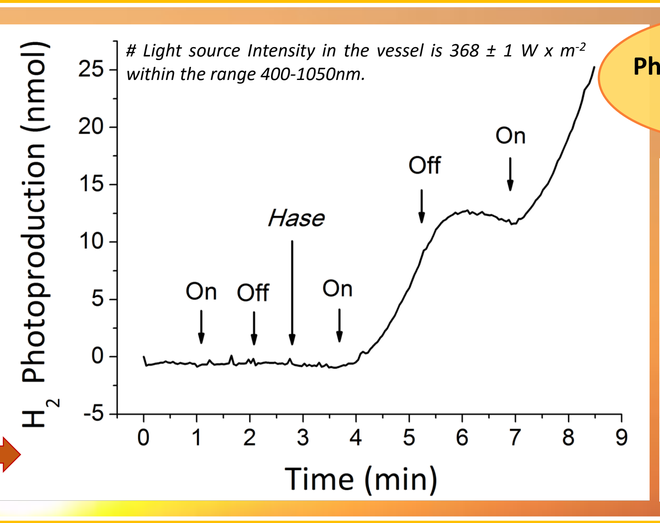
The specific area of the In_2S_3 powder measured by BET method obtained a value of 40,6±0,3 m^2/g and the average pore width was 16,5nm.
The diameter for the *Dv*-SeHase molecules is around 5nm, which favor the insertion of the enzyme into the pores during incubation process.



The hybrid In_2S_3 / Hase photocatalyst

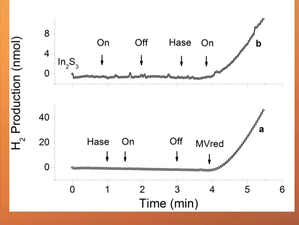
The Photocatalytic activity measurement

H_2 production is measured by mass spectrometry in 50mM Tris-HCl, pH 7, containing 0,2M sulfite, at 37°C. The Sodium sulfite was chosen as sacrificial agent.
The experiment started with the reactor containing only In_2S_3 and sulfite. The solution was irradiated from minute 1 to 2 with no production of H_2 during that time frame. Afterwards the Hase was injected inside the vessel. After 1 minute mixing the light was switched on. H_2 production was observed almost immediately. Switching off the light source interrupted the H_2 production.



Photoproduction of 292 U*

Controls
(a) without In_2S_3.
(b) without Hase.



Sulfite effect

The effect of the presence of sulfite on the specific activity of the enzyme was tested using reduced methyl viologen 1mM concentration added in the solution. The specific activity with sulfite was 1140 ± 45 U*. Whereas in absence of sulfite was 3763 ± 221 U*. Sulfite decrease 3-fold the catalytic turnover of the Hase. In_2S_3 does not affect to Hase.

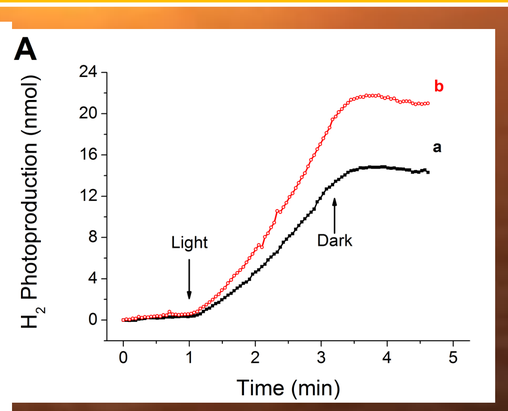
U [µmol H_2 x mg^{-1} Hase x min^{-1}]*

The incubation effect on hybrid In_2S_3 / Hase photocatalysis

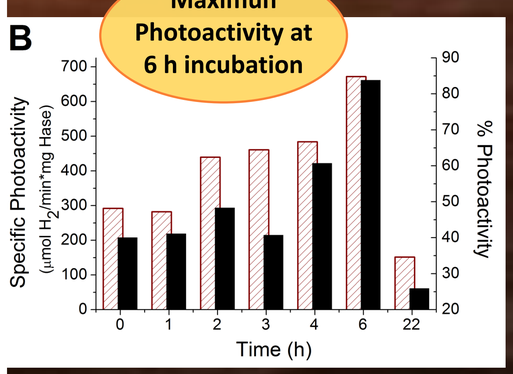
Higher photocatalytic rate after incubation of Hase with In_2S_3

Figure A shows the comparison between an experiment with no previous incubation time (a) and another after 3 hours of In_2S_3 and Hase suspension incubation at 4°C stirred at 60 rpm speed on a roller mixer. Higher photoproduction was achieved after incubation.

Figure B show the dependence of photoactivity on the incubation time (tested from 0 to 22 hours). A longer incubation yielded a higher photobioproduction of H_2, with a maximum at 6 hours incubation. The striped bars show the specific photoactivity. The black bars shown the % of photocatalytic rate for each sample compared with the measured H_2 production rate driven by reduced methyl viologen instead of light. The efficiency of the photoexcited system with no previous incubation is 40%, whereas the 6-hour incubation sample yielded 84% H_2 -photobioproduction rate efficiency.



Maximun Photoactivity at 6 h incubation

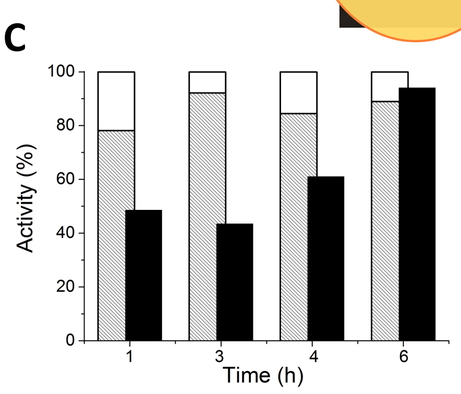


Hydrogenase inserted into the pores of the light absorber

The enzyme ratios attached to the In_2S_3 particles were measured by incubating the samples and then let to sediment under natural gravity during two hours. The supernadant liquid was then separated, and the sediment particles resuspended in fresh buffer. Figure C represents the % of H_2 production obtained with the supernadant fraction (white area in bars) and the In_2S_3 particles fraction (grey area in bars).

The black bars are the % of photoactivity in the sediment fraction compared to the Hase activity measured in the same fraction with MV. After 6 hours incubation the 89% of the Hase was attached to the In_2S_3 particles and 94% of it was photoactive.

94% of the Hase inserted into the pores of the In_2S_3 is photoactive



CONCLUSIONS

- **Photoexcited electrons that populate the In_2S_3 conductive band (CB) were transferred to the Hase's active site successfully, thus allowing the reduction of two protons to H_2.**
- **6-hours incubation sample yielded the highest H_2 – photoproduction rate efficiency: 89% of the Hase was inserted inside the pores of the In_2S_3 particles and 94% of it was photoactive.**

In_2S_3 and *D. vulgaris* [NiFeSe] hydrogenase form an efficient hybrid photocatalyst for solar H_2 production

[1] Y. Xu, M. A. A. Schoonen, *American Mineralogist*, **2000**, Vol. 85, page 543.
[2] Y. Jouanneau, B.C. Kelley, Y. Berlier, P.A. Lespinat and P.M. Vignais. *J. Bacteriol*. **1980**, Vol.143, page 628.
[3] C. Tapia, S. Zacarias, I. A. C. Pereira, J. C. Conesa, M. Pita, A. L De Lacey, *Submitted*, **2016**.

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