



**HAL**  
open science

# Augmenting the Performance of Hydrogenase for Aerobic Photocatalytic Hydrogen Evolution via Solvent Tuning

Michael G. Allan, Thomas Pichon, Jade A. McCune, Christine Cavazza, Alan  
Le Goff, Moritz F. Kühnel

► **To cite this version:**

Michael G. Allan, Thomas Pichon, Jade A. McCune, Christine Cavazza, Alan Le Goff, et al.. Augmenting the Performance of Hydrogenase for Aerobic Photocatalytic Hydrogen Evolution via Solvent Tuning. *Angewandte Chemie International Edition*, 2023, 62 (22), pp.e202219176. 10.1002/anie.202219176 . hal-04071894

**HAL Id: hal-04071894**

**<https://hal.science/hal-04071894v1>**

Submitted on 11 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

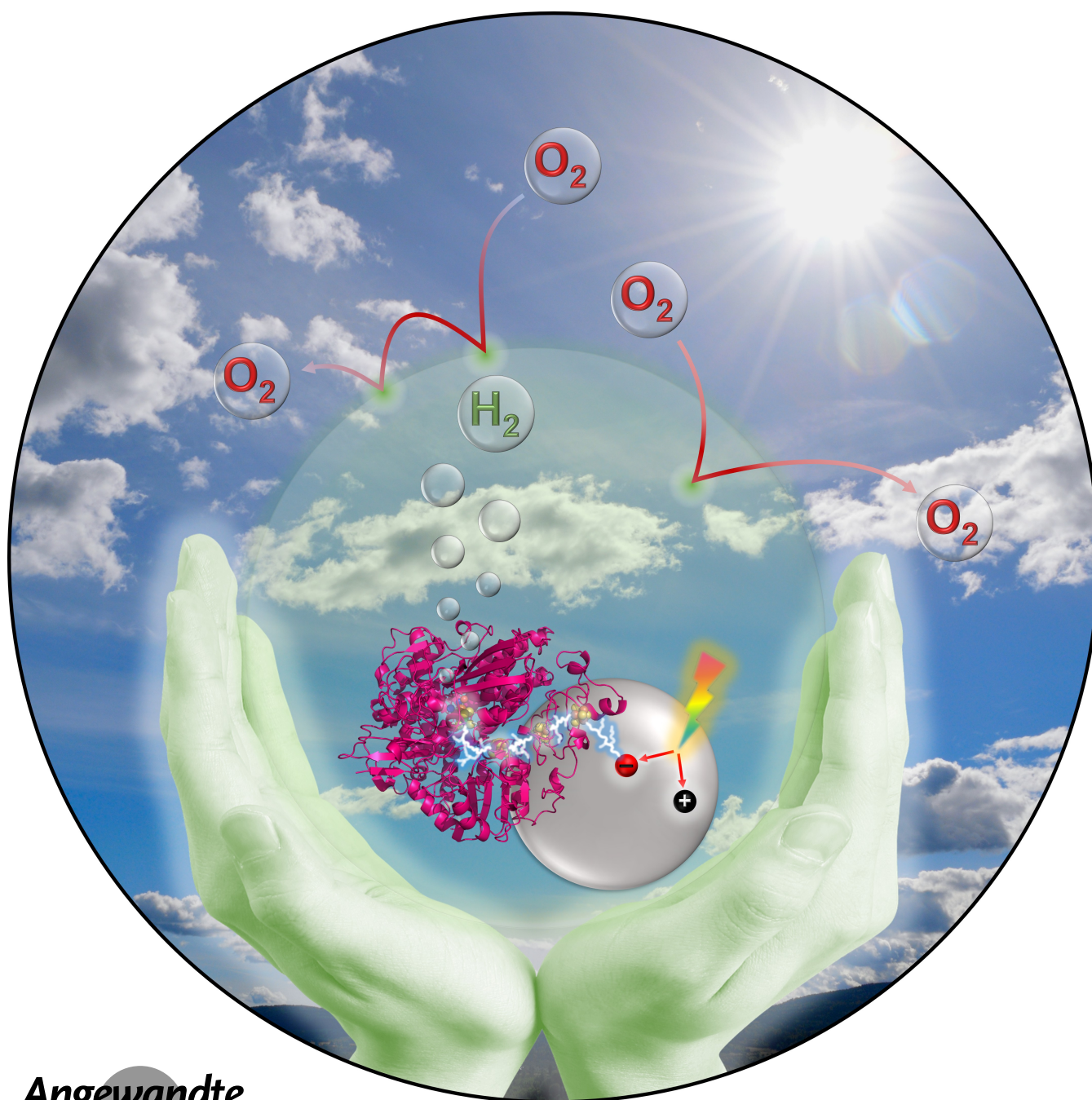
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Enzyme Catalysis

How to cite: *Angew. Chem. Int. Ed.* **2023**, *62*, e202219176

International Edition: doi.org/10.1002/anie.202219176

German Edition: doi.org/10.1002/ange.202219176

**Augmenting the Performance of Hydrogenase for Aerobic Photocatalytic Hydrogen Evolution via Solvent Tuning***Michael G. Allan, Thomas Pichon, Jade A. McCune, Christine Cavazza, Alan Le Goff, and Moritz F. Kühnel\**

**Abstract:** This work showcases the performance of [NiFeSe] hydrogenase from *Desulfomicrobium baculatum* for solar-driven hydrogen generation in a variety of organic-based deep eutectic solvents. Despite its well-known sensitivity towards air and organic solvents, the hydrogenase shows remarkable performance under an aerobic atmosphere in these solvents when paired with a TiO<sub>2</sub> photocatalyst. Tuning the water content further increases hydrogen evolution activity to a TOF of 60 ± 3 s<sup>-1</sup> and quantum yield to 2.3 ± 0.4 % under aerobic conditions, compared to a TOF of 4 s<sup>-1</sup> in a purely aqueous solvent. Contrary to common belief, this work therefore demonstrates that placing natural hydrogenases into non-natural environments can enhance their intrinsic activity beyond their natural performance, paving the way for full water splitting using hydrogenases.

Photocatalytic water splitting is viewed as a favourable method of producing green H<sub>2</sub> to combat global energy challenges without requiring large investments into electrolyzers and power grids.<sup>[1]</sup> Suitable systems for efficient solar H<sub>2</sub> production should focus on materials which are robust, cheap, and readily available.<sup>[2]</sup> A research-intensive area in this field is the development of non-precious co-catalysts for the hydrogen evolution half-reaction (HER) and its reverse hydrogen oxidation reaction (HOR).<sup>[3]</sup> [NiFe], [FeFe] and [NiFeSe] hydrogenases (H<sub>2</sub>ases) are biological catalysts which can reversibly convert protons and electrons into H<sub>2</sub> at low overpotentials without being based on precious metals.<sup>[4]</sup> Solar-driven H<sub>2</sub> generation has been demonstrated using a range of H<sub>2</sub>ase-photocatalyst combinations<sup>[5,6]</sup> with [NiFeSe] H<sub>2</sub>ases shown to be particularly active.<sup>[7]</sup> An

interesting aspect of [NiFe] and [NiFeSe] H<sub>2</sub>ases is their O<sub>2</sub> tolerance,<sup>[7g,8]</sup> whereby they show only a partial and reversible decrease in catalytic activity under aerobic conditions. Photocatalytic H<sub>2</sub> evolution in high levels of O<sub>2</sub> is an important property as catalytic components in photoreactors may be exposed to O<sub>2</sub> via in situ O<sub>2</sub> formation resulting from water oxidation or through leakage. Despite this, O<sub>2</sub>-tolerant H<sub>2</sub>ases show performances and lifetimes for H<sub>2</sub> evolution much lower in aerobic environments versus an inert environment.<sup>[7d]</sup> Solving the O<sub>2</sub> sensitivity is considered a key step towards industrial application of hydrogenases.<sup>[9]</sup>

We recently reported on a novel approach to enabling O<sub>2</sub>-tolerant H<sub>2</sub> evolution through solvent design.<sup>[10]</sup> Efficient photocatalytic H<sub>2</sub> evolution under aerobic conditions was achieved using a Pt/carbon nitride photocatalyst employing deep eutectic solvents (DESSs) with a low O<sub>2</sub> solubility and diffusivity as a reaction medium. DESSs have attracted attention in recent years as an alternative class of ionic liquids, as they possess low toxicities and can be prepared from cheap and readily available precursors.<sup>[11]</sup> However, H<sub>2</sub>ases have so far not been employed outside of conventional aqueous solvents for solar H<sub>2</sub> evolution due to incompatibility with different environments, particularly organic solvents.<sup>[12]</sup> In the past, chemical modifications to H<sub>2</sub>ases have been investigated to allow them to function in organic solvents.<sup>[13]</sup> Protection of H<sub>2</sub>ase from O<sub>2</sub> has been achieved by integration with hydrogels<sup>[14]</sup> and redox-active films.<sup>[15]</sup> In this work, we showcase the applicability of solvent engineering to the photocatalytic H<sub>2</sub> evolution at a [NiFeSe] H<sub>2</sub>ase with TiO<sub>2</sub> as a light-absorber (Figure 1). We highlight for the first time not only the stability and catalytic activity of [NiFeSe] H<sub>2</sub>ase in organic-based solvents for photocatalytic H<sub>2</sub> production, but also a remarkable enhancement in O<sub>2</sub> tolerance induced in these non-natural solvents without any enzyme modification or catalyst redesign.

[\*] Dr. M. G. Allan, Dr. M. F. Kühnel  
Department of Chemistry, Faculty of Science and Engineering,  
Swansea University  
Singleton Park, Swansea SA2 8PP, Wales (UK)  
E-mail: m.f.kuehnel@swansea.ac.uk  
Homepage: <https://moritz-kuehnel.com/>

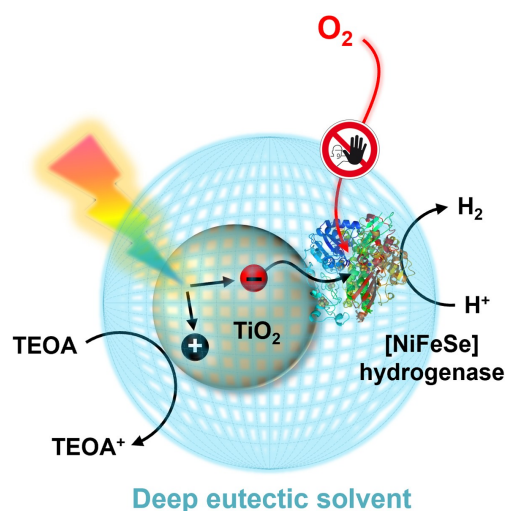
T. Pichon, Dr. C. Cavazza  
Univ. Grenoble Alpes, CEA, CNRS, IRIG, CBM  
38000 Grenoble (France)

Dr. J. A. McCune  
Melville Laboratory for Polymer Synthesis, University of Cambridge  
Lensfield Road, Cambridge, CB2 1EW (UK)

Dr. A. Le Goff  
University Grenoble Alpes, CNRS, DCM UMR 5250  
F-38000 Grenoble (France)

Dr. M. F. Kühnel  
Dept. Hydrogen Labs and Field Tests, Fraunhofer Institute for Wind  
Energy Systems  
Am Haupttor, BC 4310, 06237 Leuna (Germany)

© 2023 The Authors. Angewandte Chemie International Edition published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

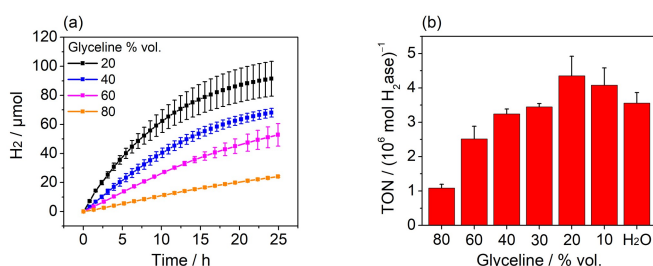


**Figure 1.** Schematic representation of the work presented here. Deep eutectic solvents induce oxygen tolerance to a TiO<sub>2</sub>-hydrogenase based photocatalyst for solar-driven hydrogen evolution under aerobic conditions.



First, we assessed the general effect of DESs on the activity of H<sub>2</sub>ases to investigate their potential for biophotocatalytic hydrogen evolution in non-conventional solvents. Previous work has shown that DESs can stabilise air-sensitive species<sup>[16]</sup> and possess a high degree of biocompatibility<sup>[17]</sup> with an ability to stabilise proteins.<sup>[18]</sup> We chose [NiFeSe] H<sub>2</sub>ase from *Desulfomicrobium baculatum* (*Db*) as a HER catalyst for this work, due to its previously reported suitability for use as a co-catalyst with various photocatalysts.<sup>[7]</sup> The photocatalytic performance for H<sub>2</sub> generation was investigated in a heterogeneous TiO<sub>2</sub>-[NiFeSe] catalyst system comprised of TiO<sub>2</sub> (2.5 mg mL<sup>-1</sup>) and *Db*[NiFeSe] H<sub>2</sub>ase (21 pmol) in a variety of DES-water mixtures. Aqueous TEOA was used as an electron donor, with the pH adjusted to 7.0 prior to mixing with the DES (Table S1). Samples were irradiated with simulated solar light (AM 1.5G) at 40 °C under a continuous purge of N<sub>2</sub>, and H<sub>2</sub> evolution was quantified by gas chromatography (see Supporting Information for full details).

In all solvents tested, we clearly observed photocatalytic H<sub>2</sub> evolution, thus proving that *Db*[NiFeSe] H<sub>2</sub>ase retains its catalytic HER activity in DES-based solvents. H<sub>2</sub> generation by the TiO<sub>2</sub>-[NiFeSe] photocatalyst system was sustained for >24 h in all solutions containing the DES glyceline (choline chloride:glycerol 1:2). The reaction rate increased upon increasing the water content in the solvents (Figure 2). In an 80 % vol. aq. glyceline solution, TiO<sub>2</sub>-[NiFeSe] generated 24.10 ± 0.55 μmol<sub>H<sub>2</sub></sub> (TON > 1080000 ± 100000) after 24.9 h irradiation whereas the same photocatalyst in a 20 % vol. aq. glyceline solution showed a TON > 4350000 ± 500000 (91.44 ± 11.96 μmol<sub>H<sub>2</sub></sub>) with an apparent quantum yield (AQE) of 2.3 ± 0.2 % (Table S2). Comparable performance was also observed in other DESs such as ethaline (choline chloride:ethylene glycol, Figure S1). This compares favourably with an aqueous solvent, in which TiO<sub>2</sub>-[NiFeSe] exhibited a TON > 3500000 ± 31000 after 24.1 h under otherwise identical conditions. Remarkably, the activity in 20 % vol. aq. glyceline exceeds that observed in a purely aqueous environment by approx. 19% under otherwise identical conditions. Negligible amounts of H<sub>2</sub> were produced when TiO<sub>2</sub> was irradiated in 60% vol. aq. glyceline

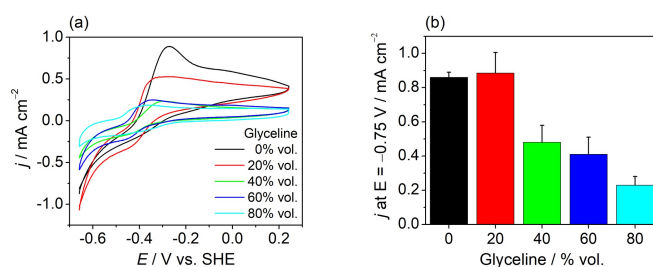


**Figure 2.** Photocatalytic H<sub>2</sub> generation using a photocatalyst system based on TiO<sub>2</sub> and *Db*[NiFeSe] H<sub>2</sub>ase in various glyceline-water mixtures. (a) H<sub>2</sub> generation over time and (b) turnover number after 24 h irradiation in solvents of varying glyceline content under inert conditions. Conditions: TiO<sub>2</sub> (5.0 mg), *Db*[NiFeSe] H<sub>2</sub>ase (21 pmol), 2.0 mL solvent, TEOA (0.4 M), AM 1.5G, 1 sun, 40 °C, constant N<sub>2</sub> purge.

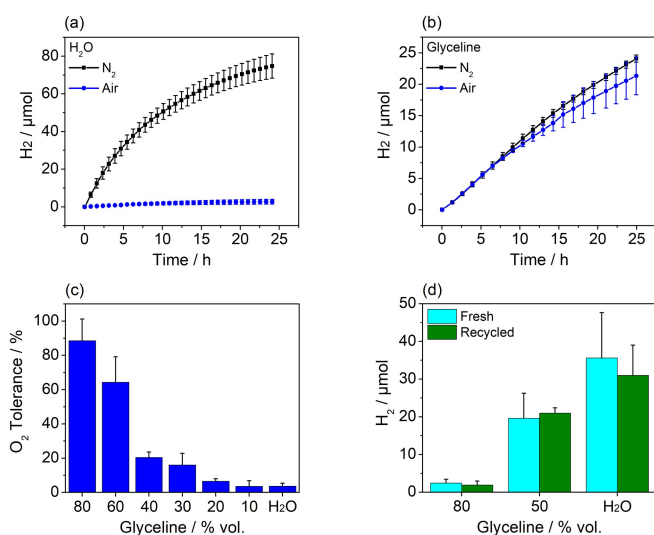
without added *Db*[NiFeSe] H<sub>2</sub>ase (Figure S2). To the best of our knowledge, this is the first report of a natural H<sub>2</sub>ase functioning in an organic solvent for solar H<sub>2</sub> production—and even slightly better than in purely aqueous conditions in which it evolved. It also exceeds most previous reports on photocatalytic H<sub>2</sub> evolution using a [NiFeSe] H<sub>2</sub>ase (Table S3).

Further insight into the origin of the varying H<sub>2</sub>ase activity depending on the DES content of the medium was sought from electrochemical measurements of *Db*[NiFeSe] H<sub>2</sub>ase adsorbed onto an adamantane-modified multi-walled carbon nanotube electrode (MWCNT)<sup>[19]</sup> in varying concentrations of glyceline. Protein film electrochemistry<sup>[20]</sup> shows that the enzyme retains its reversible activity towards both HER and HOR with near zero-overpotential in the DES (Figure 3a). Upon increasing the glyceline content in the solvent, the HER current increases before gradually decreasing (Figure 3b). The presence of an HOR current arises from the production of H<sub>2</sub> in the MWCNT layer upon proton reduction. Its decrease upon increasing the glyceline content might not only be caused by a decrease in HER activity but may be amplified by a decrease in H<sub>2</sub> solubility with higher DES concentrations, caused by the “salting out” effect of solutions with high ionic strengths.<sup>[21]</sup> A lowered H<sub>2</sub> solubility in turn would mitigate the well-documented inhibition of H<sub>2</sub>ase by H<sub>2</sub>,<sup>[22]</sup> thus allowing for a higher HER activity in DESs. The initial increase in HER current is consistent with the observed increase in photocatalytic H<sub>2</sub> generation reflecting the solvent effect on increasing the activity of the enzyme for proton reduction.

To exploit the impact of the DES content in the solvents on the stability of *Db*[NiFeSe] H<sub>2</sub>ase under demanding conditions, the TiO<sub>2</sub>-[NiFeSe] photocatalyst system was tested in the same solvents under a constant purge of air (21 % O<sub>2</sub>). In an aqueous aerobic sample, the photocatalyst produced 2.73 ± 1.3 μmol<sub>H<sub>2</sub></sub> after 24.1 h irradiation (TON = 130000 ± 60000) corresponding to only 3.7 ± 1.7 % of the amount produced under inert but otherwise identical conditions (Figure 4), owing to the known O<sub>2</sub> inhibition of H<sub>2</sub>ase. In the DES-based solutions however, a high level of oxygen tolerance is exhibited without making changes to the photocatalyst or enzyme, particularly at higher percentages



**Figure 3.** Protein film electrochemistry of *Db*[NiFeSe] H<sub>2</sub>ase adsorbed on a MWCNT electrode in glyceline-water mixtures of varying composition. (a) Cyclic voltammograms and (b) observed current density at -0.75 V vs. SHE. Conditions: *Db*[NiFeSe] H<sub>2</sub>ase-MWCNT working electrode, TEOA (0.4 M), pH 7.0, 25 °C, 0.01 V s<sup>-1</sup> scan rate, de-aerated solution.



**Figure 4.** Oxygen tolerance of the photocatalytic H<sub>2</sub> generation using a photocatalyst system based on TiO<sub>2</sub> and *Db*[NiFeSe] H<sub>2</sub>ase. (a) Photocatalytic H<sub>2</sub> generation in water and (b) in 80% vol. aq. glyceline under inert conditions and atmospheric levels of O<sub>2</sub>. (c) Oxygen tolerance of TiO<sub>2</sub>-[NiFeSe] determined from total H<sub>2</sub> produced in inert and aerobic conditions after > 24 h irradiation in solvents of varying concentrations of glyceline in water. (d) H<sub>2</sub> produced by TiO<sub>2</sub>-[NiFeSe] in fresh and resuspended solutions containing varying concentrations of glyceline after 9.3 h irradiation under N<sub>2</sub>. Conditions: TiO<sub>2</sub> (5.0 mg), *Db*[NiFeSe] H<sub>2</sub>ase (21 pmol), 2.0 mL solvent, TEOA (0.4 M), AM 1.5G, 1 sun, 40 °C, constant N<sub>2</sub> or air purge.

of DES. In an 80% vol. aq. glyceline solution, the O<sub>2</sub> tolerance was near 90% within experimental error with a total H<sub>2</sub> production of  $21.34 \pm 3.03 \mu\text{mol}_{\text{H}_2}$  (TON =  $1020000 \pm 144000$ ) after > 24 h. Similarly, the AQE of H<sub>2</sub> evolution in an aerobic 60% vol. aq. glyceline solution was determined to be  $2.3 \pm 0.4\%$  after 1 h, identical to the value determined under anaerobic conditions. After 5.2 h irradiation in air, the AQE was still at  $1.4 \pm 0.3\%$ , more than 70% of the activity in inert conditions (Table S2). While higher water contents in the solvent increased the photocatalytic activity under inert conditions (vide supra), the associated decrease in DES content lowers the O<sub>2</sub> tolerance. This decrease in O<sub>2</sub> tolerance is also observed when other DESs are used as the reaction medium (Figure S3). The optimum compromise is observed at 60% vol. aq. glyceline whereby TiO<sub>2</sub>-[NiFeSe] continuously generates H<sub>2</sub> during 72 h irradiation at a remarkable overall TOF of  $60 \pm 3 \text{ s}^{-1}$  in air, and a total TON of  $> 1800000 \pm 180000$  after the 72-h period (Figure S4). This retention of photocatalytic activity represents a major improvement over previous work showing that even O<sub>2</sub>-tolerant H<sub>2</sub>ases in water undergo considerable inactivation during photocatalytic H<sub>2</sub> evolution in the presence of air (Table S4). A [NiFe] H<sub>2</sub>ase from *D. vulgaris* shows 65% retention of activity in air vs. N<sub>2</sub> when embedded in a nanoporous glass plate.<sup>[23]</sup> In addition, an engineered [NiFe] H<sub>2</sub>ase from *E. Coli* irradiated with a carbon nitride-TiO<sub>2</sub> photocatalyst system retains 20% of its activity in air.<sup>[24]</sup> [NiFe] H<sub>2</sub>ase from *T. Roseopersicina* covalently bound to a Ru photosensitiser was reported to

maintain 11% of its initial rate in the presence of air.<sup>[5c]</sup> The *Db*[NiFeSe] H<sub>2</sub>ase used here has also been shown to exhibit photocatalytic performance in air when used with dye-sensitised TiO<sub>2</sub> as the photoabsorber in water, with a lowered H<sub>2</sub> production rate even after just 30 minutes of exposure to air.<sup>[7e]</sup>

To rationalise the dependence of O<sub>2</sub> tolerance on the solvent composition, we determined the O<sub>2</sub> solubilities  $c(\text{O}_2)$  and diffusion coefficients  $D(\text{O}_2)$  in glyceline and water using stepped-potential microwave chronoamperometry (Figure S5).<sup>[25]</sup> Using the Krichevsky<sup>[26]</sup> and Wilke-Chang<sup>[27]</sup> equations, we estimated  $c(\text{O}_2) \times D(\text{O}_2)$  for the different DES/water mixtures. Table S5 shows that as the water content increases from 0 to 100%,  $D(\text{O}_2)$  increases approx. 800-fold, while  $c(\text{O}_2)$  decreases only marginally. In line with our previously reported model for the O<sub>2</sub> tolerance in which O<sub>2</sub> intolerance is treated as diffusion-controlled O<sub>2</sub> reduction at a spherical photocatalyst particle,<sup>[10a]</sup> we observe a good correlation between  $c(\text{O}_2) \times D(\text{O}_2)$  and the O<sub>2</sub> tolerance when  $c(\text{O}_2) \times D(\text{O}_2)$  is small, i.e. when the local concentration of O<sub>2</sub> at the photocatalyst is mass transport limited and it competes with the much faster proton diffusion (Figure S6).

In addition to TiO<sub>2</sub>, photocatalytic H<sub>2</sub> evolution at *Db*[NiFeSe] H<sub>2</sub>ase was further investigated using Eosin Y (EY) as a photoabsorber in aerobic and inert conditions to test the suitability of solvent tuning on a homogeneous photocatalytic system and thus the generality of this approach. In a previous report, it has been shown that EY-*Db*[NiFeSe] is active for H<sub>2</sub> evolution under visible light irradiation in the presence of atmospheric levels of O<sub>2</sub>, however the photoreactor was not subject to resupply of O<sub>2</sub> and the activity retained in air was only 11% relative to an inert atmosphere.<sup>[7d]</sup> We observed in glyceline-based solvents that increasing the DES content led to an increase in O<sub>2</sub> tolerance of EY-*Db*[NiFeSe] H<sub>2</sub>ase (Figure S7) similar to the aforementioned heterogeneous system, however the overall activity of the photocatalyst system was low. The intrinsically low activity in DESs is in line with a previous report, where photocatalytic H<sub>2</sub> production at EY with synthetic HER catalysts was lower in DESs versus water but showed increased relative activity in air.<sup>[10a]</sup>

To investigate the origin of decreased activity at higher glyceline concentrations, solutions containing TiO<sub>2</sub> and *Db*-[NiFeSe] H<sub>2</sub>ase were subject to centrifugation, with the supernatant subsequently decanted (see Supporting Information for details). The thus obtained TiO<sub>2</sub>-[NiFeSe] pellet was resuspended in a fresh solution of the solvent in question without added H<sub>2</sub>ase and then irradiated, and the H<sub>2</sub> production was compared to a TiO<sub>2</sub>-[NiFeSe] pellet resuspended in the originally decanted supernatant. H<sub>2</sub> production performance of the TiO<sub>2</sub>-[NiFeSe] in the recycled supernatant was similar to the performance in a fresh solution (Figure 4d). This indicates that the solvent does not hinder the adsorption of the hydrogenase enzyme to the TiO<sub>2</sub> surface, allowing for efficient charge transfer from the photocatalyst to H<sub>2</sub>ase.

In summary, we have shown that DESs can act as alternative reaction media to water for the photocatalytic

hydrogen evolution using natural hydrogenase enzymes. By tuning the water content in the DESs, both activity and stability of the H<sub>2</sub> evolution activity can be increased to match and even outcompete pure water as a solvent for H<sub>2</sub> evolution. The H<sub>2</sub> evolution activity of TiO<sub>2</sub>[NiFeSe] in aerobic conditions is drastically improved in DESs, with nearly 90 % activity retained in air, whereas H<sub>2</sub> evolution in water is almost completely quenched in air. This work shows the first instance of Db[NiFeSe] H<sub>2</sub>ase employed in organic solvents for the H<sub>2</sub> evolution reaction and thus highlights the potential of solvent engineering as a novel, highly effective approach to improve natural enzyme performance. Further studies into the influence of temperature and pH on H<sub>2</sub>ase activity and O<sub>2</sub> tolerance as well as their correlation with H<sub>2</sub> and O<sub>2</sub> solubilities in the respective solvent mixtures will provide key factors for designing tailored solvents in the future that can achieve a high O<sub>2</sub> tolerance without lowering the H<sub>2</sub>ase activity.

### Acknowledgements

This work was supported by EPSRC through a DTA studentship to M.G.A. (EP/R51312X/1), and a capital investment grant to M.F.K. (EP/S017925/1), by Universities Wales through the Global Wales International Research Mobility Fund (UNIWRMF-SU/07) and by HEFCW through the Research Wales Innovation Fund Collaboration Booster. We thank Swansea University for providing start-up funds to M.F.K. and support through the Swansea-Grenoble Collaboration Fund. This work was supported by the Agence Nationale de la Recherche through the LabEx ARCANE program (ANR-11-LABX-0003-01), the Graduate School on Chemistry, Biology and Health of Univ. Grenoble Alpes CBH-EUR-GS (ANR-17-EURE-0003) and by the CFR PhD program-CEA (PhD funding for T.P.). We thank Dr. Oliver Lenz and Dr. Stefan Frielingsdorf (TU Berlin) and Prof. Frank Marken (Bath) for helpful discussions.

### Conflict of Interest

The authors declare no conflict of interest.

### Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article. Additional raw data is freely available from the Zenodo repository at <https://doi.org/10.5281/zenodo.7573371>.

**Keywords:** Deep Eutectic Solvents · Hydrogen · Hydrogenase · Oxygen Tolerance · Photocatalysis

- [1] a) A. Ajanovic, M. Sayer, R. Haas, *Int. J. Hydrogen Energy* **2022**, *47*, 24136–24154; b) Q. Wang, K. Domen, *Chem. Rev.*

- 2020**, *120*, 919–985; c) B. A. Pinaud, J. D. Benck, L. C. Seitz, A. J. Forman, Z. Chen, T. G. Deutsch, B. D. James, K. N. Baum, G. N. Baum, S. Ardo, H. Wang, E. Miller, T. F. Jaramillo, *Energy Environ. Sci.* **2013**, *6*, 1983–2002.
- [2] N. S. Lewis, D. G. Nocera, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15729–15735.
- [3] a) P. Du, R. Eisenberg, *Energy Environ. Sci.* **2012**, *5*, 6012–6021; b) S. Gao, W. Fan, Y. Liu, D. Jiang, Q. Duan, *Int. J. Hydrogen Energy* **2020**, *45*, 4305–4327; c) J. Ran, J. Zhang, J. Yu, M. Jaroniec, S. Z. Qiao, *Chem. Soc. Rev.* **2014**, *43*, 7787–7812.
- [4] a) R. Cammack, *Nature* **1999**, *397*, 214–215; b) M. Frey, *ChemBioChem* **2002**, *3*, 153–160; c) B.-E. Jugder, J. Welch, K.-F. Aguey-Zinsou, C. P. Marquis, *RSC Adv.* **2013**, *3*, 8142–8159; d) W. Lubitz, H. Ogata, O. Rüdiger, E. Reijerse, *Chem. Rev.* **2014**, *114*, 4081–4148.
- [5] a) W. Yang, G. E. Vansuch, Y. Liu, T. Jin, Q. Liu, A. Ge, M. L. K. Sanchez, D. K. Haja, M. W. W. Adams, R. B. Dyer, T. Lian, *ACS Appl. Mater. Interfaces* **2020**, *12*, 35614–35625; b) M. L. K. Sanchez, C.-H. Wu, M. W. W. Adams, R. B. Dyer, *Chem. Commun.* **2019**, *55*, 5579–5582; c) L. Zhang, S. E. Beaton, S. B. Carr, F. A. Armstrong, *Energy Environ. Sci.* **2018**, *11*, 3342–3348; d) B. Chica, C.-H. Wu, Y. Liu, M. W. W. Adams, T. Lian, R. B. Dyer, *Energy Environ. Sci.* **2017**, *10*, 2245–2255; e) O. A. Zadornyy, J. E. Lucon, R. Gerlach, N. A. Zorin, T. Douglas, T. E. Elgren, J. W. Peters, *J. Inorg. Biochem.* **2012**, *106*, 151–155; f) B. L. Greene, C. A. Joseph, M. J. Maroney, R. B. Dyer, *J. Am. Chem. Soc.* **2012**, *134*, 11108–11111; g) C. E. Lubner, R. Grimme, D. A. Bryant, J. H. Golbeck, *Biochemistry* **2010**, *49*, 404–414; h) H. Krassen, A. Schwarze, B. Friedrich, K. Ataka, O. Lenz, J. Heberle, *ACS Nano* **2009**, *3*, 4055–4061; i) I. Okura, N. Kim-Thuan, *J. Chem. Soc. Chem. Commun.* **1980**, 84b–85.
- [6] a) M. V. Pavliuk, M. Lorenzi, D. R. Morado, L. Gedda, S. Wrede, S. H. Mejias, A. Liu, M. Senger, S. Glover, K. Edwards, G. Berggren, H. Tian, *J. Am. Chem. Soc.* **2022**, *144*, 13600–13611; b) K. Holá, M. V. Pavliuk, B. Németh, P. Huang, L. Zdražil, H. Land, G. Berggren, H. Tian, *ACS Catal.* **2020**, *10*, 9943–9952; c) M. B. Wilker, J. K. Utterback, S. Greene, K. A. Brown, D. W. Mulder, P. W. King, G. Dukovic, *J. Phys. Chem. C* **2018**, *122*, 741–750; d) D. Adam, L. Bösche, L. Castañeda-Losada, M. Winkler, U.-P. Apfel, T. Happe, *ChemSusChem* **2017**, *10*, 894–902; e) M. B. Wilker, K. E. Shinopoulos, K. A. Brown, D. W. Mulder, P. W. King, G. Dukovic, *J. Am. Chem. Soc.* **2014**, *136*, 4316–4324; f) K. A. Brown, M. B. Wilker, M. Boehm, G. Dukovic, P. W. King, *J. Am. Chem. Soc.* **2012**, *134*, 5627–5636.
- [7] a) C. Tapia, S. Zacarias, I. A. C. Pereira, J. C. Conesa, M. Pita, A. L. De Lacey, *ACS Catal.* **2016**, *6*, 5691–5698; b) G. A. M. Hutton, B. Reuillard, B. C. M. Martindale, C. A. Caputo, C. W. J. Lockwood, J. N. Butt, E. Reisner, *J. Am. Chem. Soc.* **2016**, *138*, 16722–16730; c) C. A. Caputo, L. Wang, R. Beranek, E. Reisner, *Chem. Sci.* **2015**, *6*, 5690–5694; d) T. Sakai, D. Mersch, E. Reisner, *Angew. Chem. Int. Ed.* **2013**, *52*, 12313–12316; *Angew. Chem.* **2013**, *125*, 12539–12542; e) E. Reisner, D. J. Powell, C. Cavazza, J. C. Fontecilla-Camps, F. A. Armstrong, *J. Am. Chem. Soc.* **2009**, *131*, 18457–18466; f) E. Reisner, J. C. Fontecilla-Camps, F. A. Armstrong, *Chem. Commun.* **2009**, 550–552; g) C. Wombwell, C. A. Caputo, E. Reisner, *Acc. Chem. Res.* **2015**, *48*, 2858–2865.
- [8] A. Parkin, G. Goldet, C. Cavazza, J. C. Fontecilla-Camps, F. A. Armstrong, *J. Am. Chem. Soc.* **2008**, *130*, 13410–13416.
- [9] J. C. Ruth, A. M. Spormann, *ACS Catal.* **2021**, *11*, 5951–5967.
- [10] a) M. G. Allan, M. J. McKee, F. Marken, M. F. Kuehnle, *Energy Environ. Sci.* **2021**, *14*, 5523–5529; b) D. W. Wakerley, K. H. Ly, N. Kornienko, K. L. Orchard, M. F. Kuehnle, E. Reisner, *Chem. Eur. J.* **2018**, *24*, 18385–18388.

- [11] a) E. L. Smith, A. P. Abbott, K. S. Ryder, *Chem. Rev.* **2014**, *114*, 11060–11082; b) A. K. Halder, M. N. D. S. Cordeiro, *ACS Sustainable Chem. Eng.* **2019**, *7*, 10649–10660.
- [12] A. M. Klibanov, *Trends Biotechnol.* **1997**, *15*, 97–101.
- [13] C. Kim, C. A. Woodward, E. N. Kaufman, M. W. W. Adams, *Biotechnol. Bioeng.* **1999**, *65*, 108–113.
- [14] O. Ben-Zvi, I. Grinberg, A. A. Orr, D. Noy, P. Tamamis, I. Yacoby, L. Adler-Abramovich, *ACS Nano* **2021**, *15*, 6530–6539.
- [15] a) S. Hardt, S. Stapf, D. T. Filmon, J. A. Birrell, O. Rüdiger, V. Fourmond, C. Léger, N. Plumeré, *Nat. Catal.* **2021**, *4*, 251–258; b) H. Li, U. Münchberg, A. A. Oughli, D. Buesen, W. Lubitz, E. Freier, N. Plumeré, *Nat. Commun.* **2020**, *11*, 920; c) H. Li, D. Buesen, S. Dementin, C. Léger, V. Fourmond, N. Plumeré, *J. Am. Chem. Soc.* **2019**, *141*, 16734–16742; d) C. Tapia, R. D. Milton, G. Pankratova, S. D. Minter, H.-E. Åkerlund, D. Leech, A. L. De Lacey, M. Pita, L. Gorton, *ChemElectroChem* **2017**, *4*, 90–95; e) A. A. Oughli, F. Conzuelo, M. Winkler, T. Happe, W. Lubitz, W. Schuhmann, O. Rüdiger, N. Plumeré, *Angew. Chem. Int. Ed.* **2015**, *54*, 12329–12333; *Angew. Chem.* **2015**, *127*, 12506–12510.
- [16] J. A. McCune, M. F. Kuehnle, E. Reisner, O. A. Scherman, *Chem* **2020**, *6*, 1819–1830.
- [17] a) Z.-L. Huang, B.-P. Wu, Q. Wen, T.-X. Yang, Z. Yang, *J. Chem. Technol. Biotechnol.* **2014**, *89*, 1975–1981; b) J. T. Gorke, F. Srien, R. J. Kazlauskas, *Chem. Commun.* **2008**, 1235–1237.
- [18] A. Sanchez-Fernandez, S. Prevost, M. Wahlgren, *Green Chem.* **2022**, *24*, 4437–4442.
- [19] S. Gentil, S. M. Che Mansor, H. Jamet, S. Cosnier, C. Cavazza, A. Le Goff, *ACS Catal.* **2018**, *8*, 3957–3964.
- [20] F. A. Armstrong, R. M. Evans, C. F. Megarity, in *Methods Enzymol.*, Vol. 599 (Ed.: S. S. David), Academic Press, **2018**, pp. 387–407.
- [21] F. J. Millero, F. Huang, A. L. Laferiere, *Mar. Chem.* **2002**, *78*, 217–230.
- [22] V. Fourmond, C. Baffert, K. Sybirna, S. Dementin, A. Abou-Hamdan, I. Meynial-Salles, P. Soucaille, H. Bottin, C. Léger, *Chem. Commun.* **2013**, *49*, 6840–6842.
- [23] T. Noji, M. Kondo, T. Jin, T. Yazawa, H. Osuka, Y. Higuchi, M. Nango, S. Itoh, T. Dewa, *J. Phys. Chem. Lett.* **2014**, *5*, 2402–2407.
- [24] L. Zhang, G. Morello, S. B. Carr, F. A. Armstrong, *J. Am. Chem. Soc.* **2020**, *142*, 12699–12707.
- [25] J. Weber, A. J. Wain, F. Marken, *Electroanalysis* **2015**, *27*, 1829–1835.
- [26] I. R. Krichevsky, *Zh. Fiz. Khim.* **1937**, *9*, 41–47.
- [27] C. R. Wilke, P. Chang, *AIChE J.* **1955**, *1*, 264–270.

Manuscript received: December 27, 2022

Accepted manuscript online: February 14, 2023

Version of record online: March 27, 2023