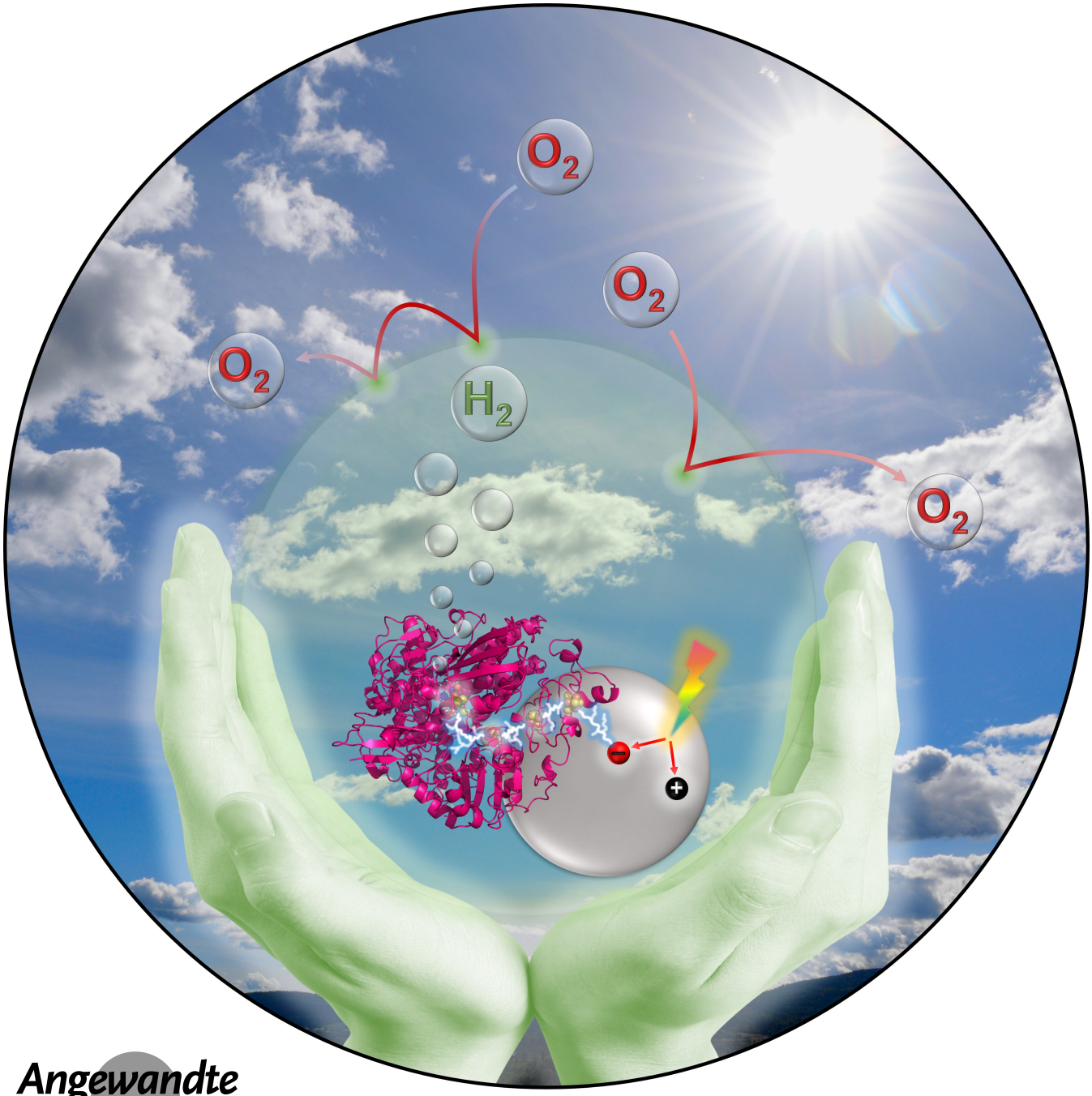
How to cite: *Angew. Chem. Int. Ed.* **2023,** *62,* e202219176
International Edition: doi.org/10.1002/anie.202219176
German Edition: doi.org/10.1002/ange.202219176

Enzyme Catalysis

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_2 photocatalyst. Tuning the water content further increases hydrogen evolution activity to a TOF of $60\pm3\,s^{-1}$ and quantum yield to $2.3\pm0.4\,\%$ under aerobic conditions, compared to a TOF of $4\,s^{-1}$ 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_2 to combat global energy challenges without requiring large investments into electrolysers and power grids.[1] Suitable systems for efficient solar H_2 production should focus on materials which are robust, cheap, and readily available.[2] 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).[3] [NiFe], [FeFe] and [NiFeSe] hydrogenases (H_2ases) are biological catalysts which can reversibly convert protons and electrons into H_2 at low overpotentials without being based on precious metals.[4] Solar-driven H_2 generation has been demonstrated using a range of H_2ase-photocatalyst combinations[5,6] with [NiFeSe] H_2ases shown to be particularly active.[7] An interesting aspect of [NiFe] and [NiFeSe] H_2ases is their O_2 tolerance,[7g,8] whereby they show only a partial and reversible decrease in catalytic activity under aerobic conditions. Photocatalytic H_2 evolution in high levels of O_2 is an important property as catalytic components in photoreactors may be exposed to O_2 via in situ O_2 formation resulting from water oxidation or through leakage. Despite this, O_2-tolerant H_2ases show performances and lifetimes for H_2 evolution much lower in aerobic environments versus an inert environment.[7d] Solving the O_2 sensitivity is considered a key step towards industrial application of hydrogenases.[9]

We recently reported on a novel approach to enabling O_2-tolerant H_2 evolution through solvent design.[10] Efficient photocatalytic H_2 evolution under aerobic conditions was achieved using a Pt/carbon nitride photocatalyst employing deep eutectic solvents (DESs) with a low O_2 solubility and diffusivity as a reaction medium. DESs 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.[11] However, H_2ases have so far not been employed outside of conventional aqueous solvents for solar H_2 evolution due to incompatibility with different environments, particularly organic solvents.[12] In the past, chemical modifications to H_2ases have been investigated to allow them to function in organic solvents.[13] Protection of H_2ase from O_2 has been achieved by integration with hydrogels[14] and redox-active films.[15] In this work, we showcase the applicability of solvent engineering to the photocatalytic H_2 evolution at a [NiFeSe] H_2ase with TiO_2 as a light-absorber (Figure 1). We highlight for the first time not only the stability and catalytic activity of [NiFeSe] H_2ase in organic-based solvents for photocatalytic H_2 production, but also a remarkable enhancement in O_2 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)

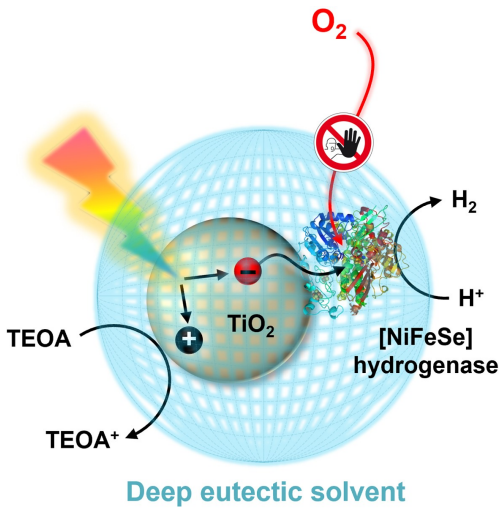


Figure 1. Schematic representation of the work presented here. Deep eutectic solvents induce oxygen tolerance to a TiO_2-hydrogenase based photocatalyst for solar-driven hydrogen evolution under aerobic conditions.

First, we assessed the general effect of DESs on the activity of H_2ases to investigate their potential for biophotocatalytic hydrogen evolution in non-conventional solvents. Previous work has shown that DESs can stabilise air-sensitive species[16] and possess a high degree of biocompatibility[17] with an ability to stabilise proteins.[18] We chose [NiFeSe] H_2ase 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.[7] The photocatalytic performance for H_2 generation was investigated in a heterogeneous TiO_2-[NiFeSe] catalyst system comprised of TiO_2 (2.5 mg mL^{-1}) and *Db*[NiFeSe] H_2ase (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_2, and H_2 evolution was quantified by gas chromatography (see Supporting Information for full details).

In all solvents tested, we clearly observed photocatalytic H_2 evolution, thus proving that *Db*[NiFeSe] H_2ase retains its catalytic HER activity in DES-based solvents. H_2 generation by the TiO_2-[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_2-[NiFeSe] generated 24.10 ± 0.55 μmol_{H2} (TON >1 080 000 ± 100 000) after 24.9 h irradiation whereas the same photocatalyst in a 20 % vol. aq. glyceline solution showed a TON >4 350 000 ± 500 000 (91.44 ± 11.96 μmol_{H2}) 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_2-[NiFeSe] exhibited a TON >3 500 000 ± 31 000 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_2 were produced when TiO_2 was irradiated in 60 %. vol. aq. glyceline without added *Db*[NiFeSe] H_2ase (Figure S2). To the best of our knowledge, this is the first report of a natural H_2ase functioning in an organic solvent for solar H_2 production—and even slightly better than in purely aqueous conditions in which it evolved. It also exceeds most previous reports on photocatalytic H_2 evolution using a [NiFeSe] H_2ase (Table S3).

Further insight into the origin of the varying H_2ase activity depending on the DES content of the medium was sought from electrochemical measurements of *Db*[NiFeSe] H_2ase adsorbed onto an adamantane-modified multi-walled carbon nanotube electrode (MWCNT)[19] in varying concentrations of glyceline. Protein film electrochemistry[20] 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_2 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_2 solubility with higher DES concentrations, caused by the "salting out" effect of solutions with high ionic strengths.[21] A lowered H_2 solubility in turn would mitigate the well-documented inhibition of H_2ase by H_2,[22] thus allowing for a higher HER activity in DESs. The initial increase in HER current is consistent with the observed increase in photocatalytic H_2 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_2ase under demanding conditions, the TiO_2-[NiFeSe] photocatalyst system was tested in the same solvents under a constant purge of air (21 % O_2). In an aqueous aerobic sample, the photocatalyst produced 2.73 ± 1.3 μmol_{H2} after 24.1 h irradiation (TON = 130 000 ± 60 000) corresponding to only 3.7 ± 1.7 % of the amount produced under inert but otherwise identical conditions (Figure 4), owing to the known O_2 inhibition of H_2ase. 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

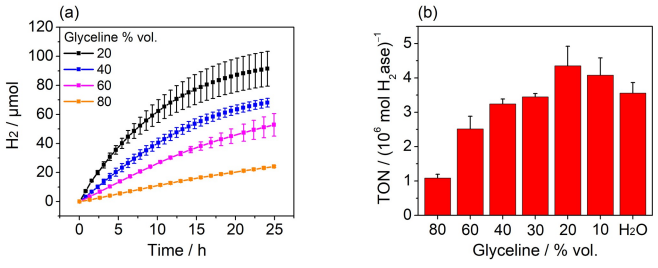


Figure 2. Photocatalytic H_2 generation using a photocatalyst system based on TiO_2 and *Db*[NiFeSe] H_2ase in various glyceline-water mixtures. (a) H_2 generation over time and (b) turnover number after 24 h irradiation in solvents of varying glyceline content under inert conditions. Conditions: TiO_2 (5.0 mg), *Db*[NiFeSe] H_2ase (21 pmol), 2.0 mL solvent, TEOA (0.4 M), AM 1.5G, 1 sun, 40 °C, constant N_2 purge.

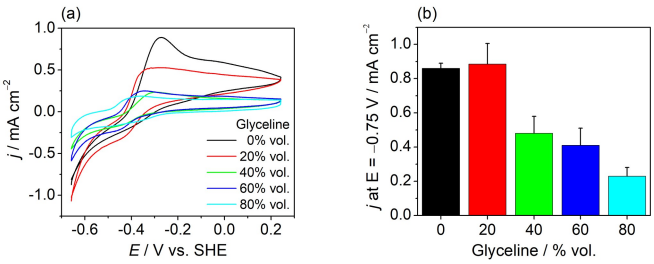


Figure 3. Protein film electrochemistry of *Db*[NiFeSe] H_2ase adsorbed on a MWCNT electrode in glyceline-water mixtures of varying composition. (a) Cyclic voltammograms and (b) observed current density at −0.75 V vs. SHE. Conditions: *Db*[NiFeSe] H_2ase-MWCNT working electrode, TEOA (0.4 M), pH 7.0, 25 °C, 0.01 V s^{-1} scan rate, de-aerated solution.

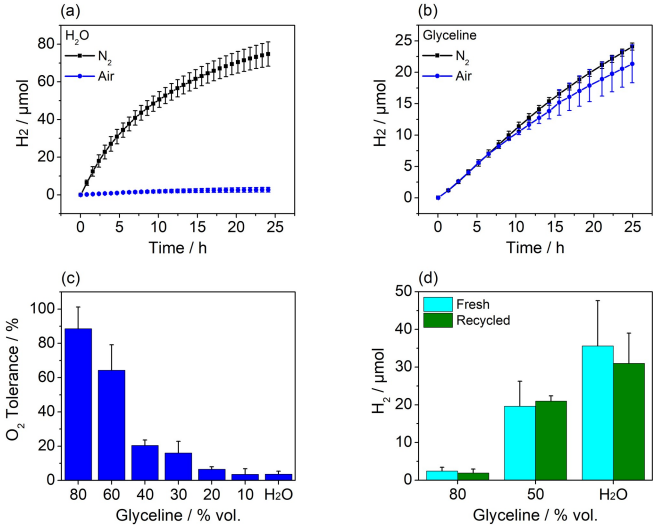


Figure 4. Oxygen tolerance of the photocatalytic H_2 generation using a photocatalyst system based on TiO_2 and *Db*[NiFeSe] H_2ase. (a) Photocatalytic H_2 generation in water and (b) in 80 % vol. aq. glyceline under inert conditions and atmospheric levels of O_2. (c) Oxygen tolerance of TiO_2-[NiFeSe] determined from total H_2 produced in inert and aerobic conditions after > 24 h irradiation in solvents of varying concentrations of glyceline in water. (d) H_2 produced by TiO_2-[NiFeSe] in fresh and resuspended solutions containing varying concentrations of glyceline after 9.3 h irradiation under N_2. Conditions: TiO_2 (5.0 mg), *Db*[NiFeSe] H_2ase (21 pmol), 2.0 mL solvent, TEOA (0.4 M), AM 1.5G, 1 sun, 40 °C, constant N_2 or air purge.

of DES. In an 80 % vol. aq. glyceline solution, the O_2 tolerance was near 90 % within experimental error with a total H_2 production of 21.34 ± 3.03 μmol_{H2} (TON = $1\,020\,000 \pm 144\,000$) after > 24 h. Similarly, the AQE of H_2 evolution in an aerobic 60 % vol. aq. glyceline solution was determined to be 2.3 ± 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 ± 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_2 tolerance. This decrease in O_2 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_2-[NiFeSe] continuously generates H_2 during 72 h irradiation at a remarkable overall TOF of 60 ± 3 s^{-1} in air, and a total TON of $> 1\,800\,000 \pm 180\,000$ after the 72-h period (Figure S4). This retention of photocatalytic activity represents a major improvement over previous work showing that even O_2-tolerant H_2ases in water undergo considerable inactivation during photocatalytic H_2 evolution in the presence of air (Table S4). A [NiFe] H_2ase from *D. vulgaris* shows 65 % retention of activity in air vs. N_2 when embedded in a nanoporous glass plate.[23] In addition, an engineered [NiFe] H_2ase from *E. Coli* irradiated with a carbon nitride-TiO_2 photocatalyst system retains 20 % of its activity in air.[24] [NiFe] H_2ase from *T. Roseopersicina* covalently bound to a Ru photosensitiser was reported to maintain 11 % of its initial rate in the presence of air.[5e] The *Db*[NiFeSe] H_2ase used here has also been shown to exhibit photocatalytic performance in air when used with dye-sensitised TiO_2 as the photoabsorber in water, with a lowered H_2 production rate even after just 30 minutes of exposure to air.[7e]

To rationalise the dependence of O_2 tolerance on the solvent composition, we determined the O_2 solubilities $c(O_2)$ and diffusion coefficients $D(O_2)$ in glyceline and water using stepped-potential microwire chronoamperometry (Figure S5).[25] Using the Krichevsky[26] and Wilke-Chang[27] equations, we estimated $c(O_2) \times D(O_2)$ for the different DES/water mixtures. Table S5 shows that as the water content increases from 0 to 100 %, $D(O_2)$ increases approx. 800-fold, while $c(O_2)$ decreases only marginally. In line with our previously reported model for the O_2 tolerance in which O_2 intolerance is treated as diffusion-controlled O_2 reduction at a spherical photocatalyst particle,[10a] we observe a good correlation between $c(O_2) \times D(O_2)$ and the O_2 tolerance when $c(O_2) \times D(O_2)$ is small, i.e. when the local concentration of O_2 at the photocatalyst is mass transport limited and it competes with the much faster proton diffusion (Figure S6).

In addition to TiO_2, photocatalytic H_2 evolution at *Db*[NiFeSe] H_2ase 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_2 evolution under visible light irradiation in the presence of atmospheric levels of O_2, however the photoreactor was not subject to resupply of O_2 and the activity retained in air was only 11 % relative to an inert atmosphere.[7d] We observed in glyceline-based solvents that increasing the DES content led to an increase in O_2 tolerance of EY-*Db*[NiFeSe] H_2ase (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_2 production at EY with synthetic HER catalysts was lower in DESs versus water but showed increased relative activity in air.[10a]

To investigate the origin of decreased activity at higher glyceline concentrations, solutions containing TiO_2 and *Db*-[NiFeSe] H_2ase were subject to centrifugation, with the supernatant subsequently decanted (see Supporting Information for details). The thus obtained TiO_2-[NiFeSe] pellet was resuspended in a fresh solution of the solvent in question without added H_2ase and then irradiated, and the H_2 production was compared to a TiO_2-[NiFeSe] pellet resuspended in the originally decanted supernatant. H_2 production performance of the TiO_2-[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_2 surface, allowing for efficient charge transfer from the photocatalyst to H_2ase.

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_2 evolution activity can be increased to match and even outcompete pure water as a solvent for H_2 evolution. The H_2 evolution activity of TiO_2-[NiFeSe] in aerobic conditions is drastically improved in DESs, with nearly 90 % activity retained in air, whereas H_2 evolution in water is almost completely quenched in air. This work shows the first instance of *Db*[NiFeSe] H_2ase employed in organic solvents for the H_2 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_2ase activity and O_2 tolerance as well as their correlation with H_2 and O_2 solubilities in the respective solvent mixtures will provide key factors for designing tailored solvents in the future that can achieve a high O_2 tolerance without lowering the H_2ase 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 (UNIW/RMF-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

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Manuscript received: December 27, 2022
Accepted manuscript online: February 14, 2023
Version of record online: March 27, 2023