A hydrogen fuel economy promises greater sustainability, lower toxicity and less greenhouse

emissions than the current fossil-fuel-based economy, provided renewable routes to hydrogen gas can be implemented. Here we use an electrolyser (Figure 1)

for electricity or light-driven water splitting to generate

only hydrogen and oxygen gases. The hydrogen gas

produced can then be used as a clean fuel to power the car (re-forming water), however in this demonstration, the hydrogen gas was collected and used to 'power' an

(Nicotinamide adenine dinucleotide) to NADH. This is an extremely important reaction in biology, and is

increasingly important for the chemical sector, because

The reaction chosen is the reduction of NAD+

Richard Avadanutei

I carried out a research project in Professor Kylie Vincent's research group across June and July 2018, to develop a self-contained Public Engagement with Research (PER) demonstration for use in classroom environments. The demonstration aims to showcase the group's research areas to a secondary school audience, particularly focusing on the hydrogen fuel economy and the use of enzymes as green catalysts for chemical synthesis. Funding for the project was provided by the University of Oxford Chemistry Department, as a Public Engagement with Research Undergraduate Bursary Award.

This project has led to development of *the first PER demonstration of the Vincent Group's catalyst system* and will contribute to the group's existing public engagement/outreach activities, under the theme of '<u>What can Chemists</u> <u>Learn from Nature</u>?'.



Figure 1: The hydrogen-fuelled car

NADH is a biological electron (energy) source. This means that NAD+ to

enzyme catalysed reaction.

NADH conversion is essential to a wide range of enzyme catalysed reduction reactions.

In this project, a combined metal-enzyme system was used, Figure 2. A metal H₂ oxidation catalyst and an enzyme NAD+ reduction catalyst are combined on carbon particles, and powered by hydrogen gas generated by the electrolyser. Advantageously, the NADH produced, is fluorescent and glows turquoise when put under a UV light source, Figure 3. The demonstration is designed to have audience participation at multiple stages, including setting up the experiment and determining the success of the experiment – *does the solution glow?*

uring the project, I tested the electrolyser set-up and developed a protocol for using the system for H₂-driven NADH production. This required development of the catalyst and the reaction conditions, as well as measuring the yield of the

reaction and considering the scale required to be able to observe fluorescence of the product. Once the viability of the set-up was confirmed, I optimised the experimental procedure by variation of the reaction parameters to develop a robust, repeatable procedure. A significant finding was that by using larger carbon particles, a complicated centrifugation step could be prevented, meaning that the entire reaction can be monitored more easily, and without the requirement for specialist equipment. I also showed that the catalyst can be premade, stored and kept under a range of conditions (room temperature, $4 \circ C$) – *showing that the demo is now suitable for school visits*.

In the future, the protocols developed here could be extended to incorporate an NADH-dependent enzyme, for a more complicated reaction. In this case, the H₂-driven NAD+/NADH reaction would be used to drive the



Figure 2: Carbon supported catalysts for H_2 oxidation and NAD⁺ reduction allow H_2 -driven NADH generation.



Figure 3: The product (NADH) fluoresces under UV

next reaction. Of particular interest would be a colour change reaction, as it would make the reaction more visible to a larger audience, without the need for a UV light source.

I then constructed a finalised experimental procedure, including a list of equipment and chemicals required, which was tested by two other members of the research group to check for clarity and reproducibility. I also created a poster to complement the experiment at PER events.

During the project I learned a variety of new practical techniques, including how to use a UV-Vis spectrophotometer as an analytical tool, a centrifuge for separation of carbon particles from solution, a benchtop UV transilluminator as a test of NAD⁺ to NADH conversion, methods for handling and using enzyme samples including use of liquid nitrogen to flash-freeze enzyme samples, a range of general biochemical techniques including preparation of buffers, micro-pipetting and other solution handling techniques and use of gas lines. I also learnt about experimental design, protocol development and keeping lab records.

Overall, the project gave me invaluable insight on how it'd be like to be a doctoral student in an actual research group, and I am very grateful to have had this experience.