Gas pressure effects on the rates of catalytic H_2 oxidation by hydrogenases – Supplementary Information

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Determination of the change in H_2 pressure in the electrochemical cell and solution concentration as a function of the gas flow rate.

To determine the effect of increasing the flow rate of gas through the electrochemical cell on the pressure in the cell, a simple manometer was incorporated into the cell design.

The manometer consisted of a length of tubing of the same diameter as the input and output of the cell, with one end connected to the gas outlet of the cell and the other left open. The tubing was bent into a 'U' shape and filled with water to a height approximately half way up the vertical 'sides' of the U. Increasing the flow rate of gas through the cell caused the pressure to increase in the headspace, and thus a 'head' of water was created, the height of which could be measured.

A simple conversion factor was used to convert the height of the head of water in cm to pressure in bar relative to atmospheric pressure (1 bar = 1020 cm (H_2O)). The overpressure in bar was then used to calculate the H_2 concentration in μM in the buffer solution, using the relationship that $c_1/p_1 = c_2/p_2$ (*i.e.*, the ratio of concentration (c) to vapour pressure (p) for a species is constant for different pressures). Solubility data (Henry's constant) were taken from *'Compilation of Henry's Law Constants for Inorganic and Organic Species of Potential Importance in Environmental Chemistry (Version 3)'* (R. Sander, 1999, accessed at http://www.henrys-law.org). As shown in Figure S1, the overpressure of H_2, and hence the H_2 concentration, increase linearly with flow rate.

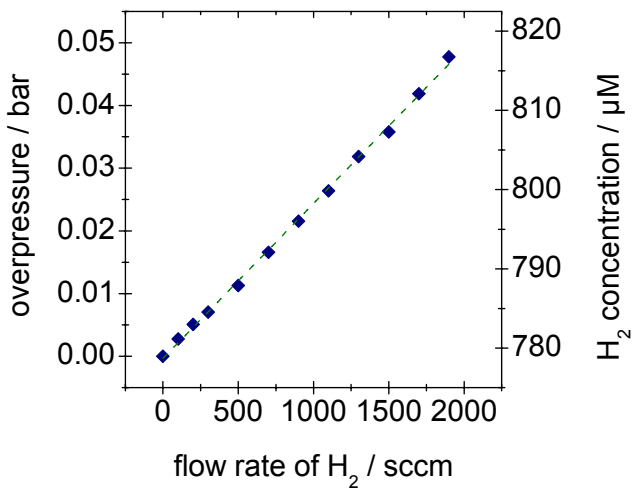

Figure S1. The relationship between the flow rate of H_2 through the cell, the gas pressure in the headspace (relative to atmospheric pressure) in bar, and the resultant H_2 concentration in solution (at 25 °C). The dashed line is the best fit to a linear equation.

This 'calibration curve' allows the flow rate in the cell to be interpreted in terms of the changes in both the gas pressure in the cell, and in the resultant H_2 concentration in solution. Crucially, the manometer also allows the rate of change in gas pressure to be measured. The mass flow controllers used to supply gas to the headspace of the cell have a response time of *ca.* 4 s, and the gas pressure in the cell, measured using the manometer, stabilizes within this time.

Examining the effect of varying electrode potential, pH and temperature

Similar experiments to those shown in Figure 1 were performed to determine how the pressure sensitivity is affected by changing the electrode potential, the pH of the electrolyte solution, and the temperature (Figure S2).

We compared the attenuation in catalytic activity at a high flow rate of H_2 (3000 sccm H_2: 0.074 bar overpressure; 838 μM H_2) with that observed at a lower flow rate (1000 sccm H_2: 0.024 bar overpressure; 799 μM H_2). In each case, all experimental variables apart from that being probed were held constant. For clarity and to account for film loss, currents were normalized to 1000 sccm; in all cases catalytic activity was diminished at the higher flow rate.

As shown in Figure S2A, there is little effect of varying the electrode potential. There is also no effect of pH (Figure S2B). However, increasing the temperature from 10 °C to 40 °C (Figure S2C) greatly increased the current attenuation at 3000 sccm, the activity dropping to 90% of its value at 1000 sccm.

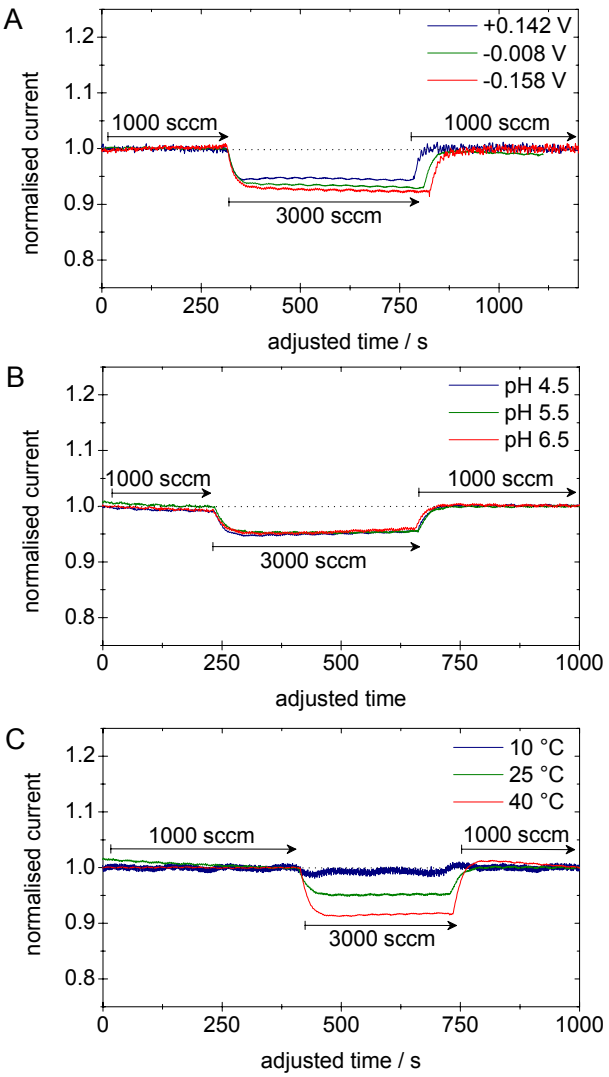

Figure S2. Experiments probing the extent of current attenuation at high flow rates of H_2 as a function of the applied electrode potential (Panel A), the buffer pH (Panel B) and the cell temperature (Panel C). In all cases, currents were normalised to the catalytic current at 1000 sccm. All experiments were performed under a gas atmosphere of 100% H_2, at a constant electrode rotation rate ω = 2500 rpm. Other conditions were as follows. Panel A: T= 25 °C, pH 5.5; Panel B: experiments were carried out at a constant overpotential η = +0.317 V *vs.* $E(H^+/H_2)$, T = 30 °C; Panel C: η = +0.317 V *vs.* $E(H^+/H_2)$, pH 5.5.