

Electricity from low-level H₂ in still air – an ultimate test for an oxygen tolerant hydrogenase

Kylie A. Vincent,^a James A. Cracknell,^a Jeremy R. Clark,^a Marcus Ludwig,^b Oliver Lenz,^b Bärbel Friedrich^b and Fraser A. Armstrong^{*a}

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We demonstrate an extreme test of O₂ tolerance for a biological hydrogen-cycling catalyst: the generation of electricity from just 3% H₂ released into still, ambient air using an open fuel cell comprising an anode modified with the unusual hydrogenase from *Ralstonia metallidurans* CH34, that oxidizes trace H₂ in atmospheric O₂, connected via a film of electrolyte to a cathode modified with the fungal O₂ reductase, laccase.

Hydrogen is often cited as the clean, 'green' fuel of the future. The enthalpy of its reaction with O₂ ($\Delta H = -286 \text{ kJ mol}^{-1} (\text{H}_2)$, 25 °C) is harnessed through combustion or, more efficiently, as electricity in fuel cells in which H₂O is the only product. However, massive efforts are needed to establish the 'hydrogen economy', including 'green' H₂ production and low-risk, cost-effective H₂ storage, transportation and utilization. Much can be learnt from biology. Reversible interconversion of H₂ and H⁺ is crucial in microbial energy cycling. H₂, primarily released in the course of anaerobic bacterial fermentation, is recovered and used as a dilute fuel by other microbes.^{1,2} These interconversions are carried out by hydrogenases, highly active metallo-enzymes with fragile and usually highly O₂-sensitive catalytic centres composed of iron ([FeFe]), or nickel and iron ([NiFe]), coordinated to the protein framework via cysteines, and to biologically unusual CO and CN ligands. Using protein film voltammetry,^{3,4} we have obtained quantitative information on [FeFe]- and [NiFe]-hydrogenases and their reactions with small inhibitory molecules such as CO and H₂S,⁵⁻⁷ as well as O₂.^{8,9} In terms of fundamental activity, their active sites compare favourably with the functional units of Pt catalysts.¹⁰

Electricity generation from H₂ and O₂ at Pt electrodes was first envisaged by Schoenbein and Grove in 1839,¹¹ and H₂ fuel cells are now an important emerging technology, with the emphasis on operation at elevated temperatures and pressures. Although Pt is an excellent electrocatalyst for both H₂ oxidation and O₂ reduction, its poor specificity for H₂ against O₂ and other small molecules (CO, H₂S) means that the anode (and high purity fuel) must be physically separated from the cathode (and oxidant), usually by a proton exchange membrane (PEM).^{12,13}

Most H₂-oxidising microbes are strict or facultative anaerobes, but so-called Knallgas bacteria are specialised in exploiting traces

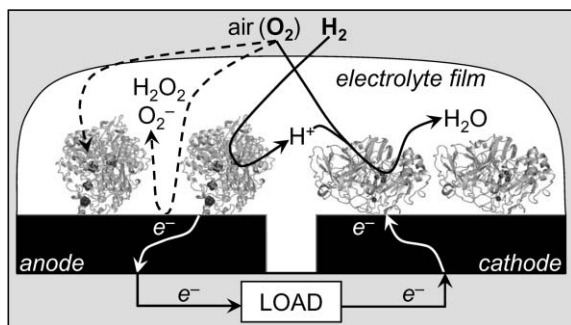
of H₂ that escape into aerobic environments. They employ hydrogenases with high catalytic selectivity and, in addition to their striking resistance towards oxidative damage, the ability to oxidise H₂ even under atmospheric O₂. In this Communication we define O₂ tolerance as the ability of a hydrogenase to actually function catalytically under O₂ as opposed to merely being able to be isolated or stored under aerobic conditions. The chemical basis of O₂ tolerance remains unclear. Well-studied Knallgas bacteria include the chemolithotrophic model organism *Ralstonia eutropha* H16,¹⁴ and the heavy metal-resistant strain *Ralstonia metallidurans* CH34.¹⁵ A key finding was that the membrane-bound [NiFe]-hydrogenase (MBH) from *R. eutropha* H16 (*Re*) was able to maintain some (ca. 20%) activity at 0.8 bar H₂, 0.2 bar O₂.⁶ This enabled us to construct a fuel cell able to function without a PEM, provided that H₂ and air were introduced separately, thus maintaining an atmosphere of close to 1 bar H₂ at the anode (modified with *Re* MBH) and 1 bar air at the cathode (modified with laccase).⁶ Isolation of the MBH from *R. metallidurans* (CH34),[†] which has been described as being even more active and stable than *Re* MBH,¹⁶ suggested an even more challenging possibility: a fuel cell that would function on fuel-poor H₂-air mixtures, a situation akin to that experienced by *Ralstonia* hydrogenases *in vivo*. In voltammetric experiments, to be described in detail elsewhere, CH34 MBH turned out to be extremely active, robust, and significantly more O₂-tolerant than *Re* MBH.

With sufficiently selective biological catalysts, power can be generated from very low levels of H₂ in air. Graphite electrodes modified‡ with CH34 MBH (anode) and laccase, a multi-copper oxidase,¹⁷ from the white rot fungus *Trametes versicolor*, formerly *Polyporus versicolor*, (cathode) were placed side by side in a shallow tray of electrolyte (100 mM citrate buffer, pH 5) to maximize gas diffusion to the surface while keeping the enzyme films wet, Scheme 1. Activities of both laccase and isolated CH34 MBH are optimal under weakly acidic conditions.

Fuel cell devices were contained within a sealed glass tank containing a stationary atmosphere of 3% H₂ in air – a non-explosive mixture¹⁸ with such a low H₂ : O₂ ratio that H₂ oxidation is a considerable challenge even for CH34 MBH.§ A single cell gave an open circuit (OC) voltage of ca. 950 mV (the thermodynamic, reversible limit is 1.23 V under standard conditions, 1 bar H₂ (anode) and 1 bar O₂ (cathode)). A maximum power density of 5.2 μW cm⁻² at 47 kΩ (11 μA cm⁻², 500 mV) was recorded. The low current density is unsurprising given the extreme dilution of fuel (equivalent to < 30 μM in aqueous solution), the low electroactive coverage of enzyme on the electrodes (below 1 pmol cm⁻² geometric area), and the likely

^aInorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, UK OX1 3QR. E-mail: fraser.armstrong@chem.ox.ac.uk; Fax: +44 1865 272690; Tel: +44 1865 272647

^bInstitut für Biologie/Mikrobiologie, Humboldt-Universität zu Berlin, Chausseestr. 117, 10115 Berlin, Germany. E-mail: baerbel.friedrich@rz.hu-berlin.de; Fax: +49 30 2093 8102; Tel: +49 30 2093 8101



Scheme 1 Reactions occurring in a fuel cell comprising a graphite anode modified with the O₂-tolerant membrane-bound hydrogenase of *R. metallidurans* CH34 and a graphite cathode modified with high potential fungal laccase in aqueous electrolyte exposed to an atmosphere of just 3% H₂ in air. The hydrogenase which oxidises H₂ to H⁺ at the anode must produce sufficient current to compete with non-productive (short-circuiting) reduction of O₂ at bare areas of the electrode, in addition to exhibiting tolerance to inhibition and damage by O₂ itself and by the reactive oxygen species O₂⁻ and H₂O₂, produced by the direct reduction of O₂.

competition, at the anode, of non-catalytic O₂ reduction with enzyme-catalyzed H₂ oxidation. Thus there is considerable scope for optimisation. Nevertheless, three cells connected in series to give a total OC voltage of 2.7 V are sufficient to power a wrist-watch for at least 24 hours (see TOC).

Various measures of cell performance are shown in Fig. 1, starting from 3% H₂ in air and measuring the OC potential. The OC potential decreases over 20 min from 0.94 to 0.88 V as the gas composition of the electrolyte (initially H₂-saturated) equilibrates with that of the environment. A variable load was connected to

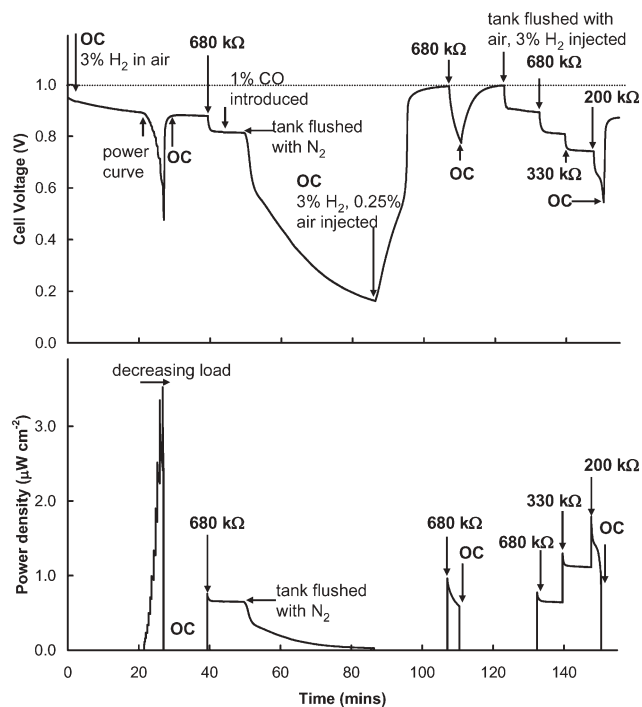


Fig. 1 Variation in fuel cell voltage and power output with time in response to changes in the gas environment or applied load for a fuel cell operated as shown in Scheme 1. OC = Open circuit.

measure a power curve; the cell voltage now drops as a current is drawn. When OC conditions are restored the voltage climbs back to 0.87 V and remains stable. After 10 min, a 680 kΩ load was applied; the cell voltage drops and then remains stable at 0.82 V, even after introduction of 1% CO. After a further 5 minutes, the tank was flushed with N₂, causing the cell voltage to drop below 0.2 V. Upon reapplying OC conditions and introduction of 3% H₂ and just 0.25% air to the N₂ atmosphere, the voltage climbs to 1 V. Under these conditions, the OC voltage is reproducibly slightly higher than the value recorded at 3% H₂ and 97% air (although the power output drops rapidly when a load is applied to draw current). After flushing the tank with air and readmitting 3% H₂, sequential decreases in load gave decreases in voltage (with increases in current). Finally, restoration of OC conditions gave 0.86 V.

Experiments with 1% H₂ in air showed that power was still produced, albeit with lower stability. Control experiments (not shown) confirmed negligible power when only one electrode was modified with enzyme, or if the O₂-sensitive *Allochromatium vinosum* hydrogenase⁹ was used in place of CH34 MBH. Power was also negligible if either H₂ or O₂ was absent from the atmosphere.

Operation of a fuel cell on a mixture of H₂ and O₂ demands catalysts that are highly selective for fuel and oxidant. Previous efforts have utilised a partially selective cathode (*e.g.*, Au, Ag, silvered Ni, SrRuO₃) to deplete O₂ from a fuel-oxidant mix before passage to a Pt anode or to enable selective O₂ reduction in a H₂-rich mixture.¹⁹ Dyer described a cell with a gas permeable membrane that produced an open circuit voltage of ≥ 950 mV when fed with a fuel-rich H₂-O₂ mixture, but the mechanism for selectivity at the electrodes (both platinum) remains unclear.^{19,20}

In our study we have pursued a rational prediction and confirmed that it is even possible to generate electricity from very low levels of H₂ in air, below the combustion limit. This is only achievable because CH34 MBH combines the attributes of high catalytic activity and a high level of discrimination for H₂ relative to O₂ without being damaged by O₂, while the laccase has high selectivity for O₂. The high affinity of the CH34 MBH for H₂ results in extremely efficient fuel conversion. As a final comment, the ability of CH34 MBH to catalyse H₂ oxidation under low H₂ concentrations perfectly reflects its physiological function in an aerobe that only occasionally encounters opportunities to use H₂.¹⁶

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Notes and references

† The CH34 MBH was overproduced in *R. eutropha* H16 using plasmid pLO6,²¹ on which the hydrogenase structural genes of H16 were exchanged by the corresponding hydrogenase genes from CH34 (GenBank accession numbers GI:94310241 and GI:94310242). A *Strep* tag II sequence was fused to the 3' end of the MBH small subunit to facilitate purification. The CH34 MBH was purified *via* affinity chromatography as previously described.⁶

‡ All solutions were prepared from purified water (Millipore, resistivity 18 MΩ cm). Pyrolytic graphite strips (1.5 cm² 'edge' surface) were fitted with insulated wires. Anodes were polished with 1 µm alumina (Buehler),

sonicated for 10 s in water and dipped in a dilute solution of CH34 MBH (ca. 0.4 mg mL⁻¹) for 30 min at 0 °C. Cathodes were abraded with SiC paper, sonicated, and dipped in a dilute solution of *T. versicolor* laccase (purified from a crude extract (Fluka) as described previously).⁶ Active enzyme on the electrodes was confirmed electrochemically in a standard three-electrode cell connected to a potentiostat (EcoChemie Autolab). Anodes were scanned between -0.35 V and 0.35 V vs. SHE under 1 bar H₂ and then immediately transferred to H₂-saturated electrolyte in the fuel cell tray. Cathodes were scanned between +0.95 V and +0.45 V under air. Under these conditions, limiting electrocatalytic current magnitudes were typically ca. 70 μA.

§ The gas atmosphere was controlled by fitting a closed 12 L glass tank with electrically conducting pins, gas inlet/outlet valves, and a septum for gas injections. The partial pressure of H₂ (Air Products) was kept below the level for combustion, e.g., an atmosphere of 3% H₂ in air was achieved by injecting the appropriate volume of H₂. Cell voltages were monitored *via* a high impedance voltmeter (Keithley 195A) or a computer interfaced with an A/D Converter and high impedance amplifier connected across the variable load (R) or device in the circuit.

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