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Hydrogen evolution catalyzed by viable and non-viable cells on biocathodes

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ABSTRACT

The presence of microorganisms on cathodes has been shown to enhance the hydrogen evolution reaction (HER), but a requirement for viable cells has not been sufficiently examined. HER was examined using live or killed biocathodes of exoelectrogenic (*Geobacter sulfurreducens*) and non-exoelectrogenic (*Escherichia coli*) bacteria, and a hydrogenotrophic methanogen (*Methanosarcina barkeri*). Electrodes at a set potential of −0.6 V (versus a standard hydrogen electrode) containing *G. sulfurreducens* biofilms or killed controls produced hydrogen at a similar rates (118 ± 15 nmold^{-1} mL^{-1}) over 5 months. Electrodes containing cell extracts produced hydrogen at approximately half this rate (56 ± 6 nmold^{-1} mL^{-1}). Biocathodes fed lactate produced only 14 ± 2 nmol/d-mL. Electrodes inoculated with *M. barkeri* produced hydrogen at a rate (120 ± 18 nmold^{-1} mL^{-1}) similar to the *G. sulfurreducens*, but no methane was recovered after the initial inoculation cycle. Non-exoelectrogenic *E. coli* cells and extracts produced hydrogen at a slower rate (13 ± 1 and 4 ± 1 nmold^{-1} mL^{-1}, over 3 cycles). Electrodes exposed to viable cells that were examined after 5 months had increased levels of in nitrogen, sulfur, iron, nickel, cobalt, and peptides (possibly remnants of hydrogenases and other oxidoreductases) relative to uninoculated controls, and no intact cells. These results show that enhanced HER can result from cell debris and that living cells are not required.

Introduction

Fossil fuels are currently the primary feedstock for hydrogen production, producing large amounts of CO_2. A sustainable alternative for hydrogen production is water electrolysis. However, water electrolyzers often use expensive precious metal catalysts to reduce electrode overpotentials. The overpotential is defined as the off-set potential needed to achieve a measurable catalytic effect compared to the theoretical potential obtained from the Nernst equation. Precious metal catalysts, such as platinum, are expensive and they are readily poisoned by trace concentrations of contaminants, such as carbon monoxide [1], alcohols [2], and sulfides [3].

Biocatalysts provide an alternative method of hydrogen production that avoids the use of precious metals. Hydrogenases are the most studied biocatalysts for hydrogen production. They are produced by microorganisms from diverse phylogenetic classifications, including methanogenic archaea [4], fermentative bacteria [5], and dissimilatory metal reducing

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bacteria [6], among others [7]. Hydrogenases are defined as oxidoreductases that have their redox potential at the same potential of the H_2/H^+ redox couple ($E = -410$ mV vs. a standard hydrogen electrode [SHE]) at ambient standard conditions (25 °C, atmospheric pressure, all concentrations 1 M except for the pH of 7). They contain an inorganic catalytic center (NiFe, FeFe, or NiFeSe [8]) surrounded by 1 or 2 protein subunits with a chain of iron sulfide clusters (4Fe–4S or 2Fe–2S) that can transfer electrons to and from the enzyme surface [9]. Purified hydrogenases have been attached to electrodes (commonly oxidized graphite) for hydrogen production [8], but the enzymes become inactive over relatively short periods of time due to protein desorption [10] or unfolding [11], especially under highly oxidative conditions [12]. Other proteins involved in electron transport, such as ferredoxins, are also able to produce hydrogen from protons and electrons provided by chemical reducing agents [13,14]. Despite being pivotal for methanogenic metabolism, hydrogen producing proteins of methanogens have not been investigated for biohydrogen production.

The use of purified enzymes is currently untenable for industrial scale hydrogen production primarily because hydrogenases are difficult to stabilize on electrodes. However, whole cells of mixed or pure cultures of actively growing microorganisms represent an economical alternative because they do not require any purification steps [15]. Pure and mixed cultures have been previously investigated for biocathodic hydrogen production. Pure culture *Geobacter sulfurreducens* biocathodes produced hydrogen at different rates over a range of potentials from −0.6 to −0.8 V (vs. SHE) [16]. Additionally, pure cultures of *Desulfovibrio* sp. grown on a cathode increased the hydrogen production rate compared to an abiotic control [17] at an electrode potential of −900 mV vs. SHE. *Desulfovibrio* sp. were also identified as the dominant species in a mixed community biocathode producing hydrogen at an electrode potential of −0.7 V vs. SHE [18]. Other recent studies observed hydrogen production using mixed culture biocathodes under mesophilic [19,20] and thermophilic [21] conditions, and using a dechlorinating consortium [22].

While the mechanism of enhanced hydrogen production by these different biocathodes is unknown, it is clear that biofilm growth on the electrode decreases overpotentials [22,23]. The presence of the microorganisms could cause a reduction in overpotential through excreted material, such as proteins that can be bound to an electrode [8], enhanced corrosion via microbial degradation of the electrode material [23,24], or an increased electron uptake by cells via unknown mechanisms [17]. Mechanisms for hydrogen production that rely only on catalytic cell materials (i.e. non-viable cells) have not been sufficiently evaluated. In addition, the long-term stability of hydrogen producing cathodes has not been well assessed. Here, the use of electrical current for hydrogen gas production with biocatalytic cell material was investigated with either live or killed pure cultures of *G. sulfurreducens*, *Methanosarcina barkeri*, or *Escherichia coli* on graphite electrodes. Reactors were run for an extended period of time (5 months) to investigate the sustainability of hydrogen production over time. Electrode surfaces were characterized spectroscopically and microscopically to identify proteins and elemental surface changes to elucidate possible reasons for enhanced hydrogen production.

Materials and methods

Reactor construction and operation

Two-chamber reactors (duplicate) were connected by side-arms (inner diameter of 2.4 cm), sealed with an O-ring, and separated by a Nafion® 117 membrane (Fuel Cell Store, Boulder, CO, USA) that was held with a screw clamp (35/25, VWR, Radnor, PA, USA). Each chamber had three 20 mm side ports, which were sealed with rubber stoppers and aluminum crimp seals, and a liquid volume of 120 mL and a headspace volume of 60 mL. A stopper was inserted into the middle side port of the working electrode chamber with a 5 mm diameter hole to allow insertion of a reference electrode.

The working electrode was made by attaching a titanium wire (0.8 mm, McMaster Carr, Cleveland, OH, USA) to a graphite block (1 cm × 2 cm) via small holes drilled near the top of the electrode. Graphite electrodes were polished with 400 and 1500 grit sandpaper, sonicated briefly to remove loose particles, and then soaked in 1 M HCl overnight. Electrodes were then rinsed three times with deionized water and polished with tissue (Kim® wipes) to remove any remaining loose material. The electrode wire was inserted through a butyl rubber stopper, placed into the top of the reactor, that was sealed with an aluminum crimp seal. A platinum wire counter electrode was inserted through a stopper in the middle side-arm in the counter electrode chamber. Electrode potentials were measured using reference electrodes (Ag/AgCl, −200 ± 5 mV vs. SHE; BASi, West Lafayette, IN, USA), and all potentials reported here are vs. SHE. All reference electrodes were replaced at the end of each batch cycle using new or refurbished electrodes. Reference electrodes were refurbished by cleaning the electrode with concentrated HCl, forming a new silver-chloride layer with a 5% hypochlorite solution, replacing the 3 M NaCl solution, and attaching a new frit. The tip (molseive, 3A, 3–4 mm diameter, Alfa Aesar, Ward Hill, MA, USA) of the inserted reference electrode was ~2 cm away from the working electrode surface. The reactors used for the incubation experiments with *Methanosarcina acetivorans* did not have side-arms in the chambers, as described in a previous study [23]. All reactors and media were sterilized before use. Media replacement was carried out using sterile, anaerobic techniques. Current uptake by the electrodes at −0.6 V was measured and recorded using a potentiostat (MPG2, Biologic Inc, Grenoble, France). Coulombic recoveries (CRs) were calculated as the ratio between measured charge transferred, and charge recovered as hydrogen or methane.

Reactors were inoculated with *G. sulfurreducens* cells and anode potentials set to −0.15 V to stimulate biofilm growth. After the biofilm developed over 14 days, the working electrode solution was changed and the potential was dropped step-wise to −0.6 V in order to examine hydrogen evolution as previously described [16]. Cycle 1 was started once the potential reached −0.6 V. Reactors containing only *M. barkeri* or *E. coli* cells, and abiotic controls, were directly set to −0.6 V. Two *G. sulfurreducens* reactors were inoculated with *M. barkeri* cells after two cycles of hydrogen production. A cycle was defined as a change in headspace hydrogen content (molar basis) of less than 10% (~2 weeks for cycles 1 and 2; ~1 month